UNIVERSIDADE FEDERAL DO PARANÁ

BRUNO ROBERTO MÜLLER

DOR EM BOVINOS NA MARCAÇÃO A FERRO QUENTE: EXPRESSÃO FACIAL, OUTROS INDICADORES COMPORTAMENTAIS E FISIOLÓGICOS E A PERCEPÇÃO DOS PRODUTORES

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Dissertação apresentada como requisito parcial à obtenção do grau de Mestre em Ciências Veterinárias no Curso de Pós-graduação em Ciências Veterinárias, Setor de Ciências Agrárias, Universidade Federal do Paraná.

Orientadora: Profa. Dra. Carla Forte Maiolino Molento

Comitê de Orientação: Prof. Dr. Ricardo Guilherme D'Otaviano de Castro Vilani e Profa. Dra. Simone Gisele de Oliveira

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A Comissão Examinadora da Defesa da Dissertação intitulada **"DOR EM BOVINOS NA MARCACÃO A FERRO QUENTE: EXPRESSAO FACIAL, OUTROS INDICADORES COMPORTAMENTAIS E FISIOLÓGICOS E A PERCEPÇÃO DOS PRODUTORES"** apresentada pelo Mestrando **BRUNO ROBERTO MÜLLER** declara ante os méritos demonstrados pelo Candidato, e de acordo com o Art. 79 da Resolução nº 65/09–CEPE/UFPR, que considerou o candidato Apto para receber o Título de Mestre em Ciências Veterinárias, na Área de Concentração em Ciências Veterinárias.

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Professora Dra. Carla Forte Maiolino Molento
Presidente/Orientadora

Professora Dra. Maria José Hötzel
Membro

Professor Dr. Ricardo Guilherme de Castro Vilani
Membro

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"…if we are to use animals for our benefit, it is morally incumbent upon us to make sure that they benefit as well, by at least living decent lives, not lives of misery, fear, and pain. To expect any less is not only immoral, it is dishonorable. It is ethically timely to use our science and technology for the benefit of the animals we use, not merely for their exploitation."

Bernard E. Rollin

RESUMO

A dor é reconhecida como uma experiência subjetiva aversiva que está relacionada com sofrimento e afeta de forma significativa o bem-estar animal. Na bovinocultura de corte, os animais são submetidos a práticas de manejo que envolvem procedimentos dolorosos, como castração, descorna, caudectomia e marcação. A marcação a ferro quente é prática comum no mundo todo e nem sempre vem acompanhada de anestesia ou analgesia. Uma das principais razões da negligência no manejo e tratamento da dor em animais de produção é a dificuldade de reconhecimento da dor. Há demanda por novos métodos de diagnóstico que sejam práticos e viáveis para aplicação em situações de campo. Assim, os objetivos deste trabalho foram explorar o potencial da expressão facial e outras medidas comportamentais e fisiológicas como indicadores de dor em bovinos e identificar a percepção de produtores sobre a marcação a ferro quente e suas consequências para o bem-estar animal. Esta dissertação foi dividida em cinco capítulos: (1) Apresentação; (2) Expressões faciais associadas à dor em bovinos de corte; (3) Diagnóstico de dor em bovinos de corte por meio de expressões faciais e outros indicadores fisiológicos e comportamentais; (4) Percepção de produtores de bovinos de corte acerca da marcação a ferro quente e suas consequências para o bem-estar animal; e (5) Considerações finais. No capítulo dois, cinco unidades de ação da expressão facial foram indicadas como potenciais indicadores de dor em bovinos: orelhas para trás, narinas dilatadas, abertura da boca e elevação medial e lateral das sobrancelhas. O capítulo três sugere que a vocalização e as cinco expressões faciais descritas no segundo capítulo desta dissertação constituem indicadores acurados e práticos no diagnóstico da dor em bovinos de corte. O capítulo quatro indica que o reconhecimento da senciência animal e da capacidade dos animais em experimentar dor não é um impedimento para mudanças nos procedimentos de identificação animal e sugere que esforços futuros devem ser concentrados em refinar e desenvolver novos métodos que sejam acessíveis e efetivos, motivando os produtores a realizar procedimentos que respeitem a qualidade de vida dos seus animais. O avanço nos métodos de diagnóstico da dor e na adoção de práticas de manejo mais compassivas interfere diretamente na vida dos animais que estão sob nossos cuidados. As conclusões apresentadas neste trabalho, se aplicadas nos sistemas produtivos, podem gerar mudanças importantes e levar a uma melhoria direta no grau de bem-estar de bovinos de corte.

Palavras-chave: bem-estar animal, expressões faciais, diagnóstico de dor, bovinocultura de corte, marcação a ferro quente

ABSTRACT

Pain is recognized as an subjective and aversive experience related to suffering, affecting significantly animal welfare. On beef cattle productive systems, animals are submitted to management practices involving painful procedures, such as castration, dehorning, tail docking and branding. Hot iron branding is a common practice adopted internationally and is not always followed by anesthesia or analgesia. One of the main causes of negligence on the management and treatment of pain in animals is the difficulty of diagnosis. There is a demand for new methods that are practically useful and viable for application in on-farm situations. Therefore, the objectives of this work were to explore the potential of facial expressions and other behavioral and physiological measurements as indicators of pain in beef cattle and identify the perception of producers on hot iron branding and its consequences to animal welfare. This thesis is divided in five chapters: (1) Presentation; (2) Facial expressions associated to pain in beef cattle; (3) Pain assessment of beef cattle using facial expressions and other physiological and behavioral indicators; (4) Perception of beef cattle producers regarding hot iron branding and its consequences to animal welfare; and (5) Final considerations. On chapter two, five facial action units were indicated as potential pain indicators in beef cattle: backwards ears, dilated nostrils, open mouth, and medial and lateral brow raise. Chapter three suggests that vocalization and the five facial expressions described on the second chapter constitute accurate and practical indicators of pain in beef cattle. Chapter four indicates that the recognition of animal sentience and the capability of animals to experience pain is not an obstacle towards changes on animal identification procedures, and suggests that future efforts should focus on refining and developing new methods that are inexpensive and effective, motivating producers to adopt procedures that are respectful to animal quality of life. The advance on pain diagnosis methods and the adoption of more compassionate management practices interfere directly on the lives of animals under our care. Conclusions presented in this work, if applied on the productive systems, may generate important changes and lead to significant improvement on the welfare of beef cattle.

Keywords: animal welfare, facial expressions, pain diagnosis, beef cattle, hot iron branding

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SUMÁRIO

1. APRESENTAÇÃO

A dor é reconhecida como uma experiência subjetiva extremamente aversiva. Está frequentemente relacionada com sofrimento, estresse e agonia (Zubieta, 2010) e é considerada como um importante indicador do estado afetivo de um animal, interferindo de forma significativa no seu grau de bem-estar (Von Keyserlingk et al., 2009). Na bovinocultura, existem diversas práticas de manejo consideradas dolorosas (Bond et al., 2012), destacando-se entre elas a marcação a ferro quente. Existe uma incoerência entre recomendações científicas e as práticas de identificação animal adotadas a campo. Estudos descrevem a marcação a ferro como um procedimento doloroso, relacionado com processos inflamatórios de longa duração (Rushen et al., 2009). Lindegaard e Andersen (2012) caracterizam a marcação a ferro como uma ferramenta pobre, ultrapassada e ineficiente e sugerem a utilização de métodos alternativos, como a implantação de microchips. Ainda assim, a marcação a ferro quente é recomendada em vários países, por exemplo, para facilitar a exportação de gado entre o Canadá e os Estados Unidos (Schwartzkopf-Genswein et al., 2012). No Brasil, a marcação de bovinos vacinados contra brucelose é obrigatória, sendo feita por meio da aplicação de um ferro quente na forma de “V” na face esquerda do animal, e sem nenhuma recomendação sobre controle e prevenção da dor (Brasil - Ministério de Agricultura Pecuária e Abastecimento, 2006).

Apesar de existir um consenso sobre os efeitos da dor sobre o bem-estar animal, é possível que os produtores não encontrem alternativas para atenuar esse problema sem consequências econômicas, levando a um conflito entre valores e interesses (Millman, 2013). Para que estratégias de controle da dor em animais sejam ativamente adotadas, é interessante que elas sejam efetivas para os animais, mas também disponíveis e em harmonia com as expectativas dos produtores (Von Keyserlingk & Hötzel, 2014; Schwartzkopf-Genswein et al., 2012).

A efetividade no diagnóstico da dor é essencial para que profissionais e produtores possam reconhecer as fontes de estímulos dolorosos nas atividades de manejo e gerar subsídio para que as intervenções e tratamentos adequados sejam realizados. Existe, entretanto, uma deficiência na capacidade atual de reconhecimento da dor (Flecknell & Roughan, 2004). Os métodos atuais utilizados não são suficientemente precisos, confiáveis ou práticos e resultam em divergências entre avaliadores (Flecknell & Roughan, 2004). A dificuldade de diagnóstico é apontada como uma das principais razões de negligência no tratamento da dor em animais (Weary et al., 2006), sugerindo uma demanda por novos métodos de diagnóstico que sejam práticos e aplicáveis no campo.

Assim, os objetivos gerais deste trabalho foram explorar medidas comportamentais e fisiológicas como indicadores de dor em bovinos, dando ênfase para a expressão facial, e identificar a percepção de produtores sobre a marcação a ferro quente e suas consequências para o bem-estar animal. Para isso, no capítulo dois, grupos musculares faciais previamente associadas à expressão facial de dor em diversas espécies foram investigados quanto à sua ativação em bovinos de corte durante um estímulo agudo de dor. Cinco características da expressão facial de bovinos apresentaram alta associação da sua ativação com a presença do estímulo doloroso, indicando potencial para serem incluídas em futuros métodos de diagnóstico de dor. No capítulo três, as cinco expressões faciais identificadas como potenciais indicadores de dor em bovinos no capítulo anterior foram avaliadas em conjunto com outras medidas fisiológicas e comportamentais de dor, com o intuito de discutir quais indicadores podem ser considerados práticos e acurados para utilização como ferramenta de diagnóstico de dor em situações de campo. Os resultados indicaram que a vocalização e a expressão facial tem tais características, podendo ser utilizadas de forma integrada em protocolos de diagnóstico de dor para bovinos. Por fim, no quarto capítulo é apresentada a percepção de produtores de gado de corte sobre a marcação a ferro quente e suas consequências para o bem-estar animal. A opinião expressa pelos produtores indica que o reconhecimento da senciência animal e da dor experimentada pelos animais que estão sob seus cuidados não é um obstáculo na direção de mudanças nos procedimentos de identificação e que esforços futuros devem ser concentrados em desenvolver novos métodos que sejam acessíveis e efetivos, motivando os produtores a realizar procedimentos que respeitem a qualidade de vida dos seus animais.

Os resultados obtidos no capítulo dois desta dissertação foram submetidos para publicação em periódico nacional Qualis A2 na área de medicina veterinária (Anexo 3). Um resumo expandido dos dados foi apresentado na forma de pôster (Anexo 5) e selecionado para apresentação oral (Anexo 4) no III Congresso Brasileiro de Bioética e Bem-estar Animal, em Agosto de 2014. Além disso, um resumo dos dados sobre vocalização de bovinos analisados no capítulo 2 também foi aceito para apresentação de pôster em um congresso internacional (Anexo 6). Os dados relativos à vocalização estão subsidiando um estudo mais detalhado desse indicador por meio do estágio curricular da aluna de graduação Karyme Zeidan (Anexo 7).

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2. EXPRESSÕES FACIAIS ASSOCIADAS À DOR EM BOVINOS DE CORTE

RESUMO

Apesar da ciência de expressões faciais em humanos estar bastante avançada, ela ainda não tem sido explorada da mesma forma em animais. O estudo de expressões faciais pode representar um avanço importante no reconhecimento e tratamento da dor em espécies ainda não estudadas. O objetivo deste trabalho foi investigar se unidades de ação (UA) faciais específicas, previamente associadas à expressão facial de dor em outras espécies, também são ativadas em bovinos de corte durante um estímulo agudo de dor. A ativação das UAs foi avaliada comparativamente através de imagens de um total de 35 bovinos de corte, antes e durante a marcação com ferro quente, caracterizando momentos sem dor (N) e com dor (P), respectivamente. Os animais observados eram 17 fêmeas e 18 machos de duas raças diferentes: Nelore e Cruzado (1/2 Nelore, 1/4 Bonsmara, 1/8 Red Angus e 1/8 Aberdeen Angus). Os resultados mostraram que não houve diferença de ativação entre machos e fêmeas, mas uma maior frequência de abertura de boca nos animais cruzados. As UAs orelhas para trás, narina dilatada, abertura de boca e elevação medial e lateral da sobrancelha apresentaram alta associação da sua ativação com a presença do estímulo doloroso, aqui representado pela marcação a ferro quente. Estas UAs devem ser consideradas no desenvolvimento de um futuro método de diagnóstico de dor que utilize a expressão facial como indicador para esta espécie.

Palavras-chave: Expressão facial, diagnóstico de dor, comportamento animal, bem-estar animal, marcação a ferro quente

ABSTRACT

Although the science of facial expression of pain in humans is very advanced, it has not been extensively explored on nonhuman animals. The study of facial expression as indicator of pain might represent a substantial advance in pain recognition and management in other species not yet studied. The objective of this work was to investigate whether specific facial action units (AU), previously related to painful facial expressions in human and some nonhuman animals, are also activated in beef cattle during acute painful stimulation. The activation of AUs was examined comparatively through pictures of a total of 35 beef cattle before and during branding with a hot iron, characterizing moments of no-pain (N) and pain (P). Animals were 17 female and 18 male beef cattle of two different genotypes: Nelore and crossbred (1/2 Nelore, 1/4 Bonsmara, 1/8 Red Angus, and 1/8 Aberdeen Angus). Results showed no differences in activation of AUs between males and females and a higher frequency of mouth opening in the crossbreed animals. The activation of the AUs backwards ears, dilated nostril, open mouth, inner brow raise, and outer brow raise was highly associated with the presence of the painful stimulus, hereby represented by hot iron branding, and should be considered on the development of further pain assessment methods using facial expressions for this species.

Key words: Facial expression, pain assessment, animal behavior, animal welfare, hot iron branding

2.1 INTRODUCTION

The facial expression has been a very effective evolutionary tool for the externalization of emotions in a wide variety of animal species (Darwin, 1872). The subtleties and meanings of the facial features in humans have been studied since the days of Aristotle (Russell, 1994) and a set of basic emotions has long been described as universally recognizable through facial expression, including happiness, surprise, fear, anger, disgust, and sadness (Duchenne, 1862). More recently, 22 categories of facial expression of emotions have been described (Du *et al.*, 2014), demonstrating the complexity of this communication model and its potential in assessing subjective feelings.

Since facial expressions are completely dependent on muscle tension and relaxation, the anatomy of facial features has been thoroughly detailed in several studies in an effort to determine the relationship between the involuntary activation of particular facial muscles and specific emotions (Ekman & Friesen, 1976; Grant, 1969). As a result of this effort, the Facial Action Coding System (FACS) was developed (Ekman & Friesen, 1978). The FACS establishes 44 fundamental anatomical components of facial movements, called Action Units (AU), allowing the description of muscles activated during a multitude of facial expressions and, therefore, of emotions (Ekman, 1993; Ekman *et al.*, 1980).

The possibility of objectively assessing emotions through facial expressions has brought a new field of work on pain research. Pain is recognized as an extremely aversive subjective experience, involving emotional components such as anger, sadness, and agony (Zubieta, 2010). The benefits of externalizing pain through facial expressions are believed to be evolutionary (Williams, 2002), and might be very effective on raising survival chances by inducing empathy in other individuals (Jackson *et al.*, 2005). The facial expressions have been shown to be consistent during the induction of pain by several modalities of nociceptive stimulation in humans, and four AU are described as comprising a basic universal signal of pain: AU4 - brow lowering, AU7 - lid tightening, AU9 – levator contraction, and AU43 - eye closure (Prkachin, 1992).

Although the science of facial expression of pain in humans is very advanced, it has not been extensively explored on nonhuman animals (Waller & Micheletta, 2013). Flecknell, 2010, discusses that this might be due to an assumption that other animals may not exhibit the same range of facial expressions as humans. This has been proved wrong by recent studies demonstrating that monkeys, sheep (Tate *et al.*, 2006) and dogs (Bloom & Friedman, 2013) may express their emotions through facial movements, and that mice (Langford *et al.*, 2010) and horses (Dalla Costa *et al.*, 2014) display specific facial expressions, activating similar AUs as humans when experiencing painful situations.

The exploration of facial expression as an indicator of pain might represent a substantial advance in pain recognition and management in other species not yet studied. It has the potential to become a very useful tool especially for use on farm animals, which are often submitted to painful procedures. The inefficiency in pain diagnosis is one of the reasons for negligence on treatment and control of management practices on farm animals, such as castration, dehorning, tail docking and branding (Weary *et al.*, 2006). Although facial expression in farm animals has received little attention, there are some evolutionary similarities with other tested animals, like the activation of analogous AUs, that are worth exploring (Millman, 2013).

The objective of this work was to investigate whether specific facial AUs, previously related to painful facial expressions in humans and other nonhuman animals, are also activated in beef cattle during acute painful stimulation.

2.2 MATERIAL AND METHODS

This experiment was approved by the Animal Use Ethics Committee of the Agricultural Sciences Campus of the Universidade Federal do Paraná (Federal University of the State of Paraná, Brazil) during session on December 16, 2013, and is registered under the protocol number 074/2013 (Anexo 1).

For this study, hot iron branding was used as a model of acute painful stimulation since it has been scientifically described as a painful procedure with long lasting inflammatory reactions and still is a common procedure used in beef cattle farms all over the world (Lindegaard & Andersen, 2012). Therefore, we selected animals from a commercial farm located in the town of Guairaçá, North of the state of Paraná, Southern Brazil. The farm was selected for reasons of proximity and presence of excellent handling facilities, and also because branding with hot iron is adopted as a standard identification procedure. No animals were branded exclusively for the purposes of this study.

We worked with a total of 35 beef cattle, 17 females and 18 castrated males. Animals weighed 209.9kg ± 33.5kg and were Nelore (20 animals) or crossbred (1/2 Nelore, 1/4 Bonsmara, 1/8 Red Angus, and 1/8 Aberdeen Angus) (15 animals). At the age of eight months, cattle were brought to the handling chute for branding, as the regular procedure on the farm. During this procedure, each animal was filmed with a digital camera (Sony SteadyShot DSC-W320) pointed to their face. Each video was one minute long and captured frames from moments before, during and after the application of the hot iron. Every time the hot iron touched the animal, a fingertip was placed in the video frame to indicate the exact moment of branding.

All videos were uploaded to a computer and frames of moments before and during the application of the hot iron were cropped using the Windows Media Player software, so each animal had a “pain” and “no-pain” picture to be investigated. All pictures were then analyzed by the same observer, according to the activation of Facial AUs. A bibliographic search was made for establishing AUs related to expression of pain, from which the following 15 were selected and analyzed in our study, in accordance to the potential of expression in beef cattle: backwards ears, characterized by the animal positioning its ears with the distal end pointed caudally (Dalla Costa *et al.*, 2014; Langford *et al.*, 2010); orbital tightening, which is the narrowing of the orbital area, with a closed eyelid (Dalla Costa *et al.*, 2014; Langford *et al.*, 2010; Prkachin, 1992); tension above the eye area, represented by the increased visibility of the underlying bone surfaces in the area above the eye (Dalla Costa *et al.*, 2014); prominent chewing muscles, characterized by the increased tension of muscles above the mouth (Dalla Costa *et al.*, 2014; Langford *et al.*, 2010); strained mouth, visible when the upper lip is drawn caudally and the lower lip is drawn cranially forming a prominent chin (Dalla Costa *et al.*, 2014); dilated nostrils, with nostrils looking strained and slightly dilated (Dalla Costa *et al.*, 2014); brow lowering, characterized by the straining of the frontal area, with eyes drawn together (Prkachin, 1992); cheek raise; represented by the convex appearance of the cheek (Langford *et al.*, 2010; Prkachin, 1992); nose wrinkle/upper lip raise, which is visible on a strained portion of skin on the bridge of the nose (Langford *et al.*, 2010; Prkachin, 1992); and open mouth (Prkachin, 1992). During the analysis of the mentioned AUs, other three were noticed to be activated when the painful stimulus was applied, and therefore were added to the study: inner brow raise and outer brow raise, characterized by the elevation and straining of medial and lateral brow area, respectively; and tongue show.

Action Units were observed comparatively and individually on pictures representing moments: “no-pain” (N) and “pain” (P). When an AU was not clearly visible it was not scored in that animal. If a determined AU was activated on both N and P frames but there was an obvious difference in intensity of activation, the less intense activation was scored as “less active” and the most intense was scored as “active” to evince the potential use of that indicator. Therefore, active AUs either represent activation or a more intensely activated AU then a “less active”.

Association between the acute painful stimulus and the activation of the AUs was determined by applying the McNemar Test. Animals with respective AU visible on only one of the N or P frames were not included in the statistical analysis. Proportions of activation of AUs between sexes and breeds on both N and P moments were tested with the Binomial Proportion Test. Statistical analysis was performed using the BioEstat 5.3 statistical software.

2.3 RESULTS

Data respective to number of animals examined and activation of AUs can be seen on Tab. 1. Only five out of 15 AUs were observable on all animals on both N and P frames: orbital tightening, tension above eye, brow lowering, eye close and inner brow raise. From these AUs, only inner brow raise presented association between painful stimulus and activation of muscle groups (P=0.0074). The AUs orbital tightening, tension above eye, brow lowering and eye closure were all not active on both N and P frames (P=1.0000).

TABLE 1. TOTAL NUMBER OF ANIMALS OBSERVED AND ACTIVATION OF THE ACTION UNITS (AU): BACKWARDS EARS (BE), ORBITAL TIGHTENING (OT), TENSION ABOVE EYE (TAE), STRAINED CHEWING MUSCLES (SCM), STRAINED MOUTH (SM), DILATED NOSTRIL (DN), BROW LOWER (BL), CHEEK RAISE (CR, NOSE WRINKLE (NW), UPPER LIP RAISE (ULR), OPEN MOUTH (OM), EYE CLOSURE (EC), INNER BROW RAISE (IBR), OUTER BROW RAISE (OBR), AND TONGUE SHOW (TS) ON ANIMALS UNDER NO-PAIN (N) AND PAIN (P) INCITEMENT.

Activation	BE	OT	TAE	SCM	SM	DN	BL	CR	NW	ULR	OM	EC	IBR	OBR	TS

of AU															
N and P	4	0	0	0	0	0	0	0	0	0	0	0	9	3	0
Only N	0	0	0	0	0	1	1	0	0	0	0	0	2	0	0
Only P	8	0	0	0	0	20	0	0	0	0	17	1	14	16	5
None	3	35	35	32	2	4	34	31	32	30	13	34	10	14	22
Total n	15	35	35	32	2	25	35	31	32	30	30	35	35	33	27

The AU backwards ears could not be examined in 20 out of 35 animals mainly due to the structure of the chute, which sometimes trapped the ears of the animals behind the neck bars, making visualization of ear position impossible. Nevertheless, statistical analysis of the remaining 15 animals with their ears visible showed high association between position of the ears and painful stimulus (P=0.0078).

In cattle, the upper lip forms an extension of the skin that covers the lower lip, hampering the visualization of these components of the AU strained mouth on this species and, therefore, impeding examination. In our experiment, this AU was observable only on two animals that had their heads in a higher position, allowing frame capture of the AU during N and P moments. Statistical association was not calculated for this AU due to the limited number of observations.

The activation of the AUs strained chewing muscle, cheek raise, nose wrinkle, and upper lip raise did not differ statistically between N and P frames (P=1.0000). On all animals observed, these AUs were inactive before and during acute painful stimulation.

The AUs dilated nostril, open mouth, and outer brow raise (Fig. 1) have all showed high statistical association with their activation and acute pain stimulation (P<0.0001). Tongue show was observed in five animals during branding, presenting a trend of association between the activation of the AU and the acute painful stimulus (P=0.0625).

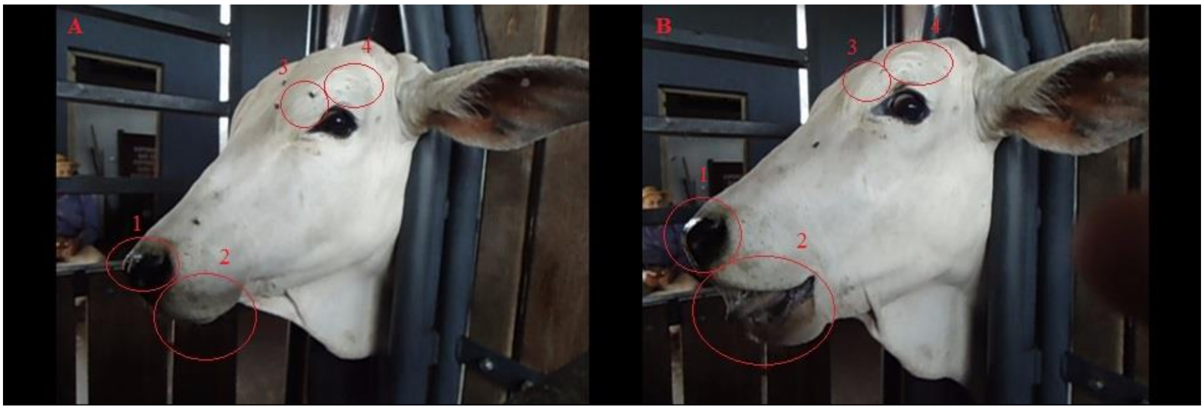

FIGURE 1. ACTION UNITS DILATED NOSTRIL (1), OPEN MOUTH (2), AND INNER (2) AND OUTER (4) BROW RAISE ON THE SAME ANIMAL MOMENTS BEFORE (A) AND DURING (B) BRANDING WITH A HOT IRON.

There was no difference on proportion of activation of AUs between male and female animals. When activation of AUs was compared between breeds, crossbred animals presented a higher proportion of animals with their mouths open when in pain (P<0.05), but no further difference was found on AU activation between breeds (Tab. 2).

TABLE 2. PROPORTION AND STATISTICAL SIGNIFICANCE (P) OF ACTIVE FACIAL ACTION UNITS ON BEEF CATTLE OF DIFFERENT SEX AND BREEDS BEFORE (NO PAIN) AND DURING BRANDING WITH HOT IRON (PAIN).

Action Units	Moment	Sex (active/observed)			Breed (active/observed)		
		Male	Female	P	Nelore	Crossbreed	P
Backwards	No Pain	2/9	2/6	0.73	2/10	2/5	0.40
Ears	Pain	6/9	6/6	0.18	7/10	5/5	0.17
Orbital	No Pain	0/18	0/17	-	0/20	0/15	-
Tightening	Pain	0/18	0/17	-	0/20	0/15	-
Tension	No Pain	0/18	0/17	-	0/20	0/15	-
Above Eye	Pain	0/18	0/17	-	0/20	0/15	-
Strained	No Pain	0/15	0/17	-	0/19	0/13	-
Chewing Muscle	Pain	0/15	0/17	-	0/19	0/13	-
Strained	No Pain	0/1	0/1	-	-	0/2	-
Mouth	Pain	0/1	0/1	-	-	0/2	-
Dilated Nostril	No Pain	1/13	0/12	0.58	1/16	0/9	0.58
	Pain	9/13	11/12	0.16	13/16	7/9	0.83
Brow Lower	No Pain	1/18	0/17	0.58	1/20	0/15	0.58
	Pain	0/18	0/17	-	0/20	0/15	-
Cheek Raise	No Pain	0/14	0/17	-	0/18	0/13	-
	Pain	0/14	0/17	-	0/18	0/13	-
Nose Wrinkle	No Pain	0/15	0/17	-	0/19	0/13	-

	Pain	0/15	0/17	-	0/19	0/13	-
Upper Lip	No Pain	0/14	0/16	-	0/18	0/12	-
Raise	Pain	0/14	0/16	-	0/18	0/12	-
Open Mouth	No Pain	0/14	0/16	-	0/18	0/12	-
	Pain	6/14	11/16	0.15	7/18	10/12	0.02
Eyes Close	No Pain	0/18	0/17	-	0/20	0/15	-
	Pain	1/18	0/17	0.58	1/20	0/15	0.58
Inner Brow	No Pain	6/18	5/17	0.80	5/20	6/15	0.34
Raise	Pain	11/18	12/17	0.55	13/20	10/15	0.92
Outer Brow	No Pain	2/16	1/17	0.50	2/19	1/14	0.74
Raise	Pain	9/16	10/17	0.88	11/19	8/14	0.97
Tongue Show	No Pain	0/14	0/13	-	0/17	0/10	-
	Pain	4/14	1/13	0.11	3/17	2/10	0.88

2.4 DISCUSSION

Hot iron branding was chosen as a model for acute painful stimulation in our study because it has long been related to increased escape-avoidance reaction as well as increased heart rate and increased epinephrine release in beef cattle, indicating great acute pain sensation (Lay *et al.*, 1992). Similarly to our results, Watts & Stookey also found that the application of a hot branding iron to beef cattle yielded a much higher rate of vocal response than a sham branding treatment (Watts & Stookey, 1999). In our study, we measured only the facial expression responses to pain and, for an improved and systematic assessment, it would also be interesting to consider the correlation with other behavioral and physiological indicators on further investigations.

The difficulty of access with the camera in the chute to film the face of the animals and also the constant movement of their head during branding resulted in a reduced number of clear images available for evaluation. This differed from other studies where animals were filmed hours after surgical intervention and images were clearer and more easily obtained (Dalla Costa *et al.*, 2014). Acute pain is known to increase head shaking behavior (Heinrich *et al.*, 2010) and general activity (Millman, 2013) of cattle, all of which interfere negatively with filming. However, as our objective was to evaluate acute pain responses, video capture had to occur at the same moment as the painful provocation, not afterwards. Recognition of acute responses to pain may be of great value to animal welfare as it permits rapid management of pain and reduces the duration of suffering (Flecknell & Roughan, 2004), especially for those animals reared extensively without close care.

Nonetheless, the number of pictures investigated in our study is very similar to other studies that have successfully identified and described AUs related to pain in other species (Dalla Costa *et al.*, 2014; Keating *et al.*, 2012; Langford *et al.*, 2010).

Some of the AUs previously related to pain expression in other species (Dalla Costa *et al.*, 2014; Keating *et al.*, 2012; Langford *et al.*, 2010; Prkachin, 1992), such as orbital tightening, tension above eye, strained chewing muscle, strained mouth, brow lower, cheek raise, nose wrinkle, upper lip raise, eye close, and tongue show have not shown a pain specific response in our experiment. This might be explained by evolutionary reasons, where it might not be functionally interesting for a prey animal like cattle to show a big range of pain expressions to a predator (Davidson *et al.*, 2002). Additionally, behaviors in response to pain vary greatly between species and this also includes facial expressions. Similar facial expressions might express distinct emotions depending on the species, so care must be taken when interpreting them (Waller & Micheletta, 2013).

Five of the AUs studied showed high association with their activation and the acute pain caused by branding: backwards ears, dilated nostril, open mouth, and inner and outer brow raise. The AUs backwards ears and dilated nostril have been studied before in other animal species and proved to be reliable pain indicators in mice submitted to a 0.9% acetic acid abdominal constriction test (Langford *et al.*, 2010), in rabbits undergoing ear tattooing (Keating *et al.*, 2012), and in horses after surgical castration (Dalla Costa *et al.*, 2014). The AU open mouth has not been described as a pain indicator in animals, but is intensely activated when humans experience shock and cold pain assays (Prkachin, 1992). The opening of the mouth might also be related to vocalization, which also increases in frequency when cattle are under pain (Watts & Stookey, 2000). Further investigation using images together with audio should help determining the possible relationships. To our knowledge, the other two AUs associated with pain in our study, inner and outer brow raise, have never been reported as pain indicators in facial expression studies before. This might be explained by inter-specific differences explained earlier, but also by the fact that, different from our experiment, all studies have focused in responses to pain up to eight hours after animal stimulation (Dalla Costa *et al.*, 2014), not on immediate responses to acute pain. Inner and outer brow raising have been related to the expression of other emotions, such as fear and surprise, in studies with humans (Du *et al.*, 2014; Williams, 2002). In fact, the sudden onset and the early stages of pain might produce a compounded experience of pain and startle that could culminate in this facial expression (Prkachin, 1992).

The description of the five specific AUs related to acute pain identified in this study is of great value for the development of new methods of pain assessment using facial expressions in cattle and might, consequently, impact positively the welfare of these animals (Flecknell, 2010). The establishment of new pain assessment methods that are non-invasive, low cost, and practical could allow us to manage animal pain far more effectively than it is possible today (Flecknell & Roughan, 2004). Assessment of pain through facial expression seems to comprise all of these criteria and should be more broadly explored for application in cattle and other farm animal species.

2.4 CONCLUSION

The activation of the AUs backwards ears, dilated nostril, open mouth, inner brow raise, and outer brow raise in beef cattle was highly associated with the presence of an acute painful stimulus, hereby represented by hot iron branding, and should be considered on the development of further pain assessment methods using facial expressions for this species.

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3. PAIN ASSESSMENT OF BEEF CATTLE USING FACIAL EXPRESSIONS AND OTHER PHYSIOLOGICAL AND BEHAVIOURAL INDICATORS

ABSTRACT

Difficulties in communication between humans and animals are a problem when it comes to animal pain assessment. The aim of this study was to measure a series of physiological and behavioural indicators with emphasis on facial expressions of pain in cattle during the practice of hot iron branding, investigating what indicators could be considered practical and accurate to be used as a diagnosis tool on field situations. We analysed plasma cortisol levels, heart and respiratory rates, escape attempts, tail wagging, vocalization and facial expressions of 70 beef cattle from a commercial farm in southern Brazil, which regularly employs hot iron branding in their animals. Animals were separated in two groups: 35 hot branded (HB) and 35 sham branded animals (SB). Results showed no statistical differences on results of cortisol, heart and respiratory rates, escape attempts and tail wagging between groups. Proportion of animals vocalizing as well as number of vocalizations per animal was significantly higher in the HB group when compared to SB. Latency for the first vocalization was significantly lower for the animals on the HB group. All five facial expressions analysed in the study presented differences in proportion of activation between treatments, with a higher proportion of animals displaying specific facial characteristics during effective branding when compared to animals that experienced the sham procedure. Animals being branded also displayed a more complex combination of facial expressions than animals sham branded. Measures of cortisol, heart and respiratory rates, escape attempts and tail wagging did not seem informative about the pain status of cattle during branding. Vocalization and facial expressions seem to constitute precise and practical indicators of pain, with potential to be included on in-farm pain assessment protocols for cattle.

Key words: Animal welfare, animal behaviour, facial expression, pain diagnosis, hot iron branding

3.1 INTRODUCTION

In human medicine, pain is commonly diagnosed based on the reported feelings that the patient himself verbally declares to be experiencing. Whenever verbal communication is not possible, as in the case of new-born children or impaired people, the assessment of pain becomes more complicated and must rely on other methods of diagnosis (Anand, 2001; Epps, 2001).

Difficulties in communication between humans and animals are a problem when it comes to animal pain assessment. Veterinarians still face difficulties when assessing animal’s pain, and there is room for improvement in the teaching of the relevance of pain to animal welfare at Veterinary Medicine programmes (Borges, 2010). Results from a study conducted by Hugonnard et al. (2004) show that identifying painful procedures is one of the main difficulties faced by veterinarians when handling the animals under their care. Since animals are not able to communicate verbally, assessment of pain often depends on observation of physiological and behavioural indicators. Physiologically, cortisol concentration is a well stablished parameter for identification of stressful situations and painful procedures. Elevated concentrations of this hormone have been related to practices such as hot iron disbudding in dairy calves (Stilwell et al., 2010), hot iron branding in horses and cattle (Erber et al., 2012; Lay et al., 1992), castration without anaesthesia in piglets (Kluivers-Poodt et al., 2012), disbudding in goats (Alvarez et al., 2015), and several other practices in multiple species (Mormède et al., 2007). Behavioural indicators of pain might include withdrawal responses and attempts to escape (Millman, 2013), vocalization (Watts & Stookey, 2000), restlessness and movement of limbs close to the source of stimuli (Weary et al., 2006), and many other species specific responses (Sneddon et al., 2014).

Unfortunately, many of these indicators are not practical or objective, especially for application at farm settings (Weary et al., 2006). Besides, none of these indicators should be interpreted separately, on their own, as they might not precisely reflect the real pain status of the animal (Bateson, 1991). Therefore, efforts should be made to develop new reliable, multi-criteria, and practically useful assessment methods that would enable us to manage pain more effectively (Flecknell & Roughan, 2004).

The use of facial expressions as indicators of pain has been recently explored in animals, and it counts with several positive aspects, being low cost, non-invasive and applicable at field situations (Flecknell, 2010). Langford et al., 2010, have developed a pain scale based on mice facial expressions, the Mouse Grimace Scale. Similarly, a scale of pain has also been created based on the facial expressions of rabbits (Keating et al., 2012), and more recently a method of pain assessment was developed based on the facial expression of horses (Dalla Costa et al., 2014). Additionally, in a pilot study in 2014, our research group identified five facial expressions associated to painful stimuli in cattle (Müller et al., 2014). The study of facial expressions of pain have originated with humans (Duchenne, 1862; Ekman & Friesen, 1978), but wherever there are similarities in anatomy, animal equivalents to some of the human facial expressions of pain are worth researching (Millman, 2013).

The aim of this study was to measure a series of physiological and behavioural indicators with emphasis on facial expressions of pain in cattle during the practice of hot iron branding in a commercial beef farm, investigating what indicators could be considered practical and accurate to be used as a diagnosis tool on field situations.

3.2 MATERIAL AND METHODS

This experiment was approved by the Ethics Committee for Animal Use of the Agricultural Sciences Campus of the Universidade Federal do Paraná (Federal University of the State of Paraná, Brazil) during session on December 16, 2013, and is registered under the protocol number 074/2013 (Anexo 1).

For this study, we considered hot iron branding a model for acute painful stimulation. Branding with a hot iron has been scientifically described as a painful procedure with long lasting inflammatory reactions (Rushen et al., 2009). However, it is still common practice among beef cattle farmers all over the world (Lindegaard & Andersen, 2012) and also in southern Brazil. Therefore, the animals used for this experiment were all from a commercial farm located in the town of Guairaçá, North of the state of Paraná, Southern Brazil. The farm was selected for reasons of proximity and presence of handling facilities compatible with the needs of our experiment, and also because branding with hot iron was adopted as a standard identification procedure. No animals were branded exclusively for the purposes of this study.

We worked with 70 animals, 34 female and 36 castrated male. Animals weighed 209.0±30.1kg and were Nelore (n=39) or crossbred (1/2 Nelore, 1/4 Bonsmara, 1/8 Red Angus, and 1/8 Aberdeen Angus, n=31) cattle. At the age of eight months, animals were brought to the handling chute for branding, as the regular procedure at the farm. Animals entered one by one in the chute and were assigned to one of the two experimental groups alternately, the first going to the group of animals that would be hot branded (HB), and the second going to the group that would go through the same handling process, except that branding irons were not hot, characterizing sham branding (SB).

Animals entered the chute and waited 5 min inside until they were branded. After branding, they waited another 10 min until released to a succeeding chute. In the following chute, animals remained for 15 min and were then directed to another subsequent chute where they stayed for more 15 min. This process repeated until each animal had passed through four consecutive chutes. This procedure was adopted to enable the detection of physiological value curves. After the last chute, animals that were hot branded returned to pasture and animals that went through the sham branding were kept in a paddock for later actual branding, so as to maintain the regular identification procedures of the farm.

3.2.1 Physiological data collection

After animals entered the first chute, a blood sample was collected by jugular venepuncture for analysis of levels of cortisol before the branding procedure. Immediately after blood collection, heart and respiratory rates were recorded. Still on the first chute, after 11 +- 1 min, another blood sample was collected and heart and respiratory rates recorded. Animals were then released to the second chute.

From the second chute until the fourth, blood samples and heart and respiratory rates were collected near the end of the 15 min period each animal stayed inside each chute. By adopting this sampling regimen, we obtained physiological data for each animal in five different periods: 5 min prior branding, and 10, 25, 40, and 55 min after branding. This allowed us to study the physiological curves with more detail in the case of any significant differences between treatments.

Blood samples (5 mL) were taken by jugular venepuncture and immediately centrifuged for 10 min at 2000 rpm. The plasma was removed and frozen at -20°C until assay. Plasma cortisol concentrations were determined by ELISA. A preliminary cortisol analysis was performed on a quota of 20 random animals, 10 from each group, to identify any differences between treatments and the need of further analysis. Blood samples analysed on this preliminary assessment were respective to 5 min before and 25 min after branding, when cortisol level differences should be most evident.

3.2.2 Behavioural data collection

For behavioural assessment, we measured the frequency and intensity of attempts to escape the chute, tail wagging, and vocalization. Each of these behaviours was observed from the moment of branding until 1 min after the procedure. Behaviours such as jumping, kicking, and head bumping inside the chute were considered attempts to escape. For the observation of attempts to escape and tail wagging, animals were filmed with a digital camera (GoPro Hero2) pointed to the left side of the first chute, where branding took place. To assess vocalization, we used the same videos from the facial expression analysis. All videos were uploaded to a computer and analysed by the same observer.

3.2.3 Facial expression analysis

During branding, each animal was filmed with a digital camera (Sony SteadyShot DSC-W320) pointed to their face. Each video was 1 min long and captured frames from moments before, during and after the procedure. Every time the iron touched the animal, a fingertip was placed in the video frame to indicate the exact moment of branding.

Frames from the moment when the branding iron touched the animals were cropped from the videos using the Windows Media Player software, so each animal had a picture of its facial expression during branding to be investigated. All pictures were analysed by the same observer, according to the activation of five facial expressions, previously associated with painful stimuli on a pilot study: backwards ears, characterized by the animal positioning its ears with the distal end pointed caudally; dilated nostrils, with nostrils looking strained and slightly dilated; inner and outer brow raise, characterized by the elevation and straining of medial and lateral brow area; and open mouth.

The facial expressions were observed individually and compared between pictures from animals experiencing pain from the hot iron branding (HB) and animals going through the sham procedure (SB). When a specific facial expression was not clearly visible, it was not scored for that animal.

3.2.4 Statistical Analysis

Statistical analyses were performed using the BioEstat 5.0 software. Prior to comparison of physiological and behavioural data between groups, a normality test was performed using Shapiro-Wilk. For comparison between treatments, we used analysis of variance for parametric data and Mann-Whitney for non-parametric data. For comparison of physiological values throughout the five periods of data collection, we used Student's t-test for paired samples. The Binomial test was used to compare proportion of animals performing specific behaviours between treatments. Finally, we used Mann-Whitney to compare the number of facial expressions displayed simultaneously by animals between groups.

3.3 RESULTS

3.3.1 Cortisol, Heart and Respiratory Rates

Plasma cortisol levels did not show any significant differences between treatments on the preliminary analysis, thus further measurements on other samples was not continued. The mean cortisol levels at 5 min before branding were 55.0 ± 29.6 nmol/L for the HB group, and 60.2 ± 24.7 nmol/L for the SB group (P=0.68). Compared to values obtained before branding, levels of cortisol at 25 min after branding increased (P<0.01) on average 22.2 ± 27.3 nmol/L for the HB group, and 23.2 ± 19.1 nmol/L for the SB group, with no significant differences on cortisol level increment between groups (P=0.91). Mean cortisol levels at 25 min after branding were 77.2 ± 25.1 nmol/L for the HB group, and 83.5 ± 26.3 nmol/L for the SB group (P=0.60). Changes in plasma cortisol are presented in Fig. 2.

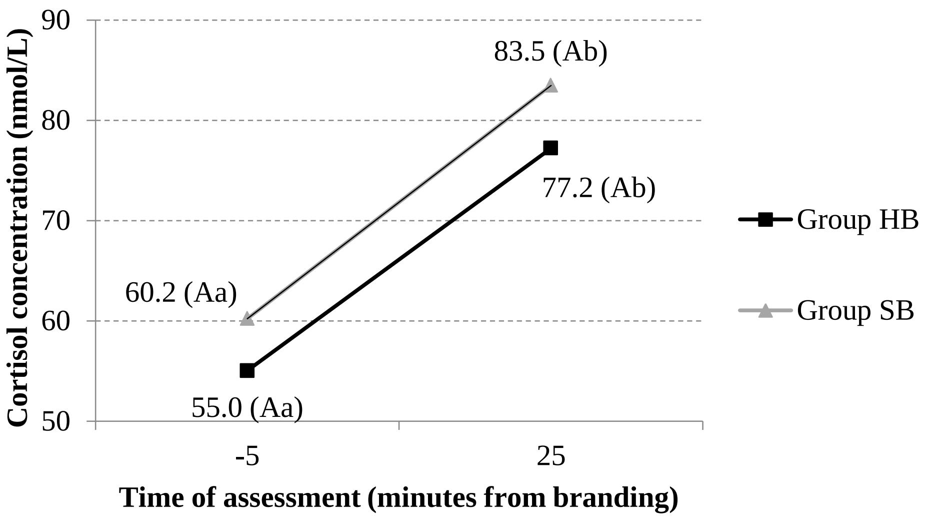

FIGURE 2. MEAN CORTISOL CONCENTRATION OF 20 BEEF CATTLE HOT BRANDED (HB) AND SHAM BRANDED (SB) IN SOUTHERN BRAZIL, 2014, AT 5 MIN BEFORE AND 20 MIN AFTER THE BRANDING PROCEDURE. MEAN VALUES FOLLOWED BY DIFFERENT UPPER CASE LETTERS REPRESENT SIGNIFICANT DIFFERENCES BETWEEN TREATMENTS (P<0.05). MEAN VALUES FOLLOWED BY DIFFERENT LOWER CASE LETTERS REPRESENT SIGNIFICANT DIFFERENCES BETWEEN TIMES OF ASSESSMENT (P<0.05).

Heart rates did not show any significant effect of treatment (P=0.14), but there was an effect of time of assessment, with values decreasing after the first assessment (P<0.01). There was no interaction between treatment and time of assessment (P=0.10). Mean heart rates were 115.43 ± 26.73, 104.00 ± 21.71, 81.60 ± 18.18, 96.57 ± 17.87, and 85.14 ± 22.74 beats/min for HB, and 118.29 ± 24.68, 107.77 ± 20.67, 65.60 ± 11.32, 93.94 ± 16.19, and 84.91 ± 22.23 beats/min for SB, at -5, 10, 25, 40, and 55 min from branding, respectively.

Respiratory rates have also not shown any significant effect of treatment (P=0.21) or interaction between treatment and time of assessment (P=0.35). There was, however, an effect of time of assessment, with values decreasing after the first assessment (P<0.05). Mean values of respiratory rates were 53.37 ± 19.67, 52.46 ± 16.63, 56.57 ± 15.83, 50.29 ± 15.74, and 44.46 ± 14.77 breaths/min for HB, and 49.26 ± 13.88, 49.60 ± 12.99, 51.54 ± 12.79, 49.26 ± 13.68, and 48.00 ± 15.28 for SB, at -5, 10, 25, 40, and 55 min from branding, respectively. Results of heart and respiratory rates can be found on Fig. 3.

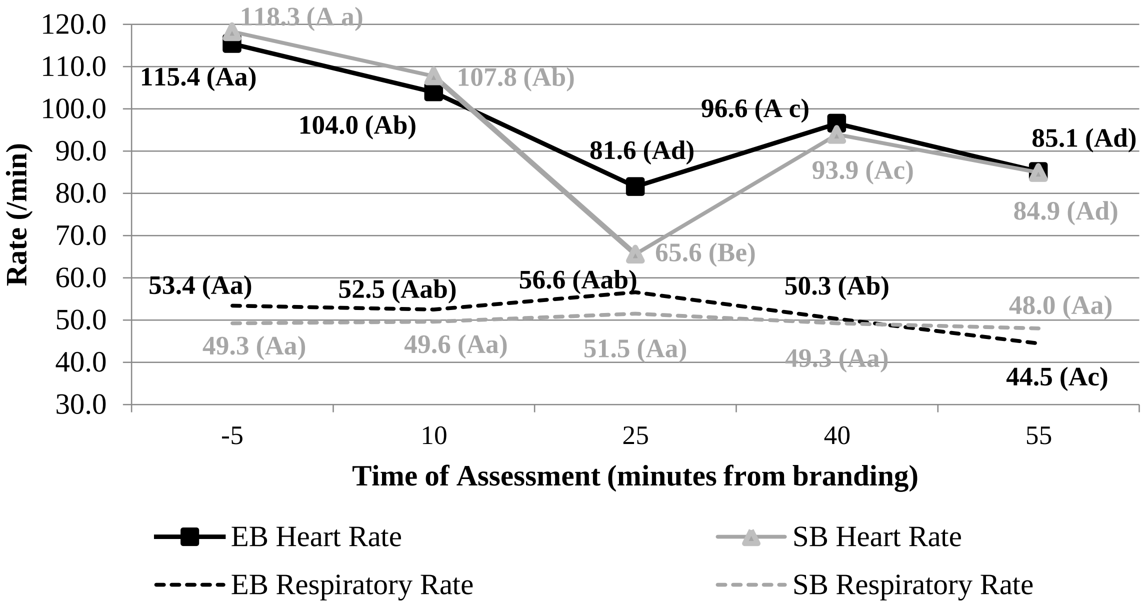

FIGURE 3. MEAN HEART AND RESPIRATORY RATES OF 70 BEEF CATTLE EXPOSED TO HOT BRANDING (HB) AND SHAM BRANDING (SB) PROCEDURES, IN SOUTHERN BRAZIL, 2014. MEAN VALUES FOLLOWED BY DIFFERENT UPPER CASE LETTERS REPRESENT SIGNIFICANT DIFFERENCES BETWEEN TREATMENTS (P<0.05). MEAN VALUES FOLLOWED BY DIFFERENT LOWER CASE LETTERS REPRESENT SIGNIFICANT DIFFERENCES BETWEEN TIMES OF ASSESSMENT (P<0.05).

3.3.2 Escape attempts, tail wagging, and vocalization

Due to problems occurred during file transfer from camera to computer, behavioural video data from two animals of each group was lost. Thus, video analysis of escape attempts and tail wagging was done for a total of 66 animals, 33 in each group.

The proportion of animals that attempted to escape was not significantly different between groups (*P*=0.13), with a total of 19 HB and 13 SB animals. Median number of attempts to escape were 1.0 (minimum 0.0, maximum 3.0) attempts for group HB, and 0.0 (0.0 – 4.0) attempts for group SB, with no statistical differences between groups (*P*=0.28). The mean duration of attempts to escape was not different between groups either (*P*=0.98), with median values of 2.7 (1.0 – 10.0) s for HB and 2.5 (1.0 – 7.0) s for SB. Median latency time from branding to first attempt of escape was 0.0 (0.0 – 55.0) s for group HB, and 7.0 (0.0 – 51.0) s for group SB, with no statistical differences between groups (*P*=0.24).

The proportion of animals that wagged their tails during the period of observation was similar between groups (*P*=0.11), with a total of 26 HB and 20 SB animals. The median number of tail wags was similar between groups (*P*=0.75), with 4.0 (4.0 – 68.0) wags for group HB, and 3.0 (0.0 – 50.0) wags for group SB. The mean intensity of movements was 0.9 (0.4 – 2.1) wags/s for group HB, and 0.9 (0.6 – 1.6) wags/s for group SB, with no statistical differences between groups (*P*=0.62). The latency until the first tail wag was also similar for both groups (*P*=0.53), with median values of 0.0 (0.0 – 40.0) s for HB, and 2.0 (0.0 – 41.0) s for SB.

The proportion of animals vocalizing was significantly higher (*P*<0.01) for HB (26/35 animals) when compared to SB (10/35 animals). Number of vocalizations per animal during the time of observation was also significantly higher for HB (*P*<0.01), with a median of 1.0 (0.0 – 18.0) vocalizations, and 0.0 (0.0 – 13.0) vocalization for SB. Mean duration of each vocalization was not different between groups (*P*=0.47), with vocalizations lasting a median of 1.0 (0.5 – 1.1) s on group HB, and 1.0 (0.0 – 1.0) s on group SB. Latency for the first vocalization showed a trend to lower values on group HB (*P*=0.06), with animals vocalizing at a median of 1.0 (0.0 – 38.0) s after branding on group HB and 5.0 (0.0 – 42.0) s on group SB. Data of vocalization can be found on Fig. 4.

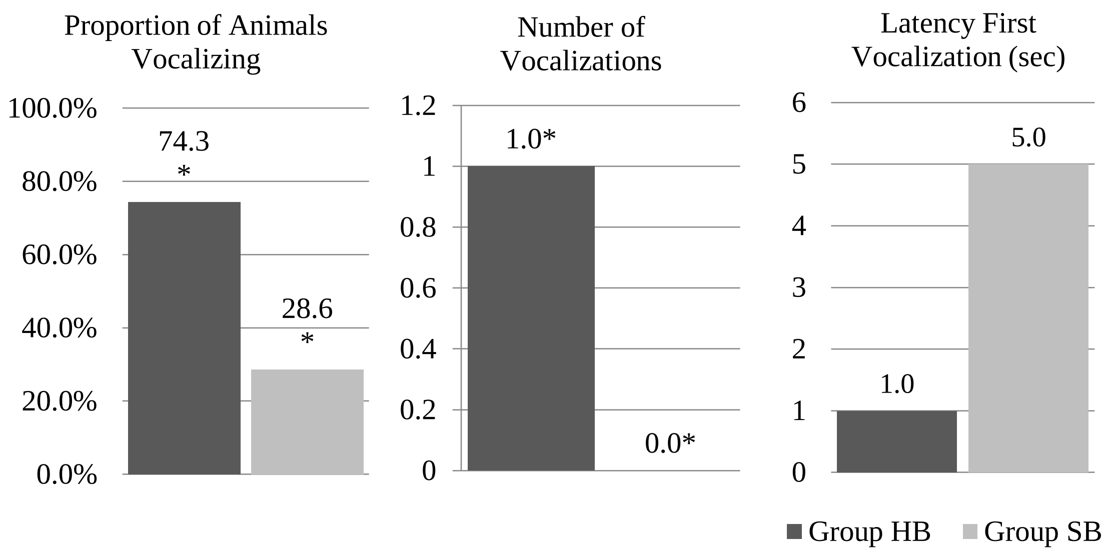

FIGURE 4. PROPORTION OF ANIMALS VOCALIZING, MEDIAN NUMBER OF VOCALIZATIONS, AND MEDIAN LATENCY FOR THE FIRST VOCALIZATION OF BEEF CATTLE HOT BRANDED (HB) AND SHAM BRANDED (SB) IN A COMMERCIAL FARM IN SOUTHERN BRAZIL, 2014. VALUES FOLLOWED BY * PRESENT STATISTICAL DIFFERENCES BETWEEN GROUPS (P<0.05).

3.3.3 Facial Expressions

Due to limited access with the camera in the chute, some characteristics of the facial expression in a number of animals were not visible in the videos. This resulted in different numbers of animals in each characteristic of facial expression for each group. Data respective to number of animals examined for the five facial expressions in each group can be seen on Tab. 3.

TABLE 3. TOTAL NUMBER OF OBSERVATIONS OF SPECIFIC FACIAL FEATURES AND NUMBER OF FACIAL EXPRESSIONS PRESENT DURING HOT BRANDING (HB) AND SHAM BRANDING (SB) IN SOUTHERN BRAZIL, 2014. SPECIFIC FACIAL EXPRESSION FOLLOWED BY AN * PRESENT STATISTICAL DIFFERENCES BETWEEN GROUPS.

Group	Condition observed	Specific facial expressions				
		Backwards Ears*	Dilated Nostrils*	Open Mouth*	Inner Brow Raise*	Outer Brow Raise*
HB	Total (Present + Absent)	16	26	31	35	33
	Present, n (%)	13 (81%)	21 (81%)	18 (58%)	23 (66%)	19 (58%)
SB	Total (Present + Absent)	30	29	31	35	35
	Present, n (%)	4 (13%)	12 (41%)	0 (0%)	6 (17%)	6 (17%)

All five facial expressions analysed presented differences in proportion of activation between treatments, with a higher proportion of animals displaying the specific facial characteristics during effective branding when compared to animals that experienced the sham procedure (P<0.01). For backwards ears, the number of animals displaying this characteristic was 13 out of 16 observed animals on group HB, and 4 out of 30 animals observed on group SB. For dilated nostrils, the proportion of activation was 21/26 for HB, and 12/29 for SB. The mouth was open in 18/31 HB pictures and in 0/31 SB pictures. For inner brow raise, we were able to analyse all 35 animals from each group and whereas 23 animals from group HB showed activation of this facial expression, only six animals from group SB displayed the same behaviour. Similarly, the proportion of animals displaying outer brow raise was 19/33 for HB, and 6/35 for SB.

Hot branded animals displayed a more complex combination of facial expressions than animals sham branded (P<0.01). Hot branded animals displayed a mode of 3 (min 0 - max 4) combined facial expressions, and sham branded animals displayed a mode of 0 (0 - 3) combined facial expressions. The proportion of animals activating none of the facial expressions analysed during branding was significantly different between groups (*P*<0.01), with 1/35 HB animals, and 17/35 SB animals.

Hot branded animals also displayed a more complex combination of observed behaviours than sham branded animals (*P*<0.01). Hot branded animals displayed a mode of 6 (1 – 7) combined behaviours, including vocalization, attempt to escape, tail wag, and each one of the five facial expressions observed, while sham branded animals displayed a mode of 3 (0 – 5) combined behaviours. The proportion of animals displaying more than one of the observed behaviours was also significantly different between treatments (*P*<0.01), with 32/33 HB animals and 20/33 SB animals.

3.4 DISCUSSION

Although there was a significant increase in cortisol levels from before either HB or SB to 25 min after the procedure, no physiological data showed differences between hot branded and sham branded animals. This was not expected, since animals experiencing painful stimulation tend to show higher plasma cortisol concentrations (Molony et al., 1995) and higher respiratory and heart rates (Coetzee, 2011; Stock et al., 2013) when compared to control animals. Plasma cortisol concentrations of cattle generally show significant increases at around 10-15 min after the onset of an aversive stimulus (Mormède et al., 2007), and peak concentrations can reach mean values of 94.8 nmol/L when animals are disbudded with hot iron without proper anaesthesia (Stilwell et al., 2010). Also, pain activates stress response systems, which increase heart and respiratory rates almost immediately to prepare the animal for what is known as the “fight or flight” response (Schwartzkopf-Genswein et al., 2012). Heart rates increase significantly 5 min after disbudding and may remain above baseline levels for more than 20 min if the procedure is done without local anaesthetics (Stewart et al., 2008).

On the other hand, despite the expectation that physiological values would significantly increase in response to the acute painful stimulus, there might be other explanations for the similarity on results from both groups. The mere handling of the animals is acknowledged to cause physiological changes that may mask and lead to underestimates of the effects of more invasive treatments (Mellor et al., 2000). The restraining of the animals during the experiment may have caused a “ceiling effect” on cortisol levels and heart and respiratory rates. This effect has been described by Molony and Kent (1997) on both physiological and behavioural responses in castrated lambs. Similarly to our results, Lay et al. (1992) have also found that handling and restraining caused an increase in heart rates and observed no differences between cortisol concentrations after branding and during restraining of cattle. Animals castrated by different methods apparently do not show any significant differences on levels of cortisol when compared to those of a control group, also probably because of the restraining needed during the procedure (Becker et al., 2012).

In fact, compared to baseline, our results showed elevated values of all physiological responses already at the pre-treatment assessments, indicating that the ceiling effect might have originated earlier, when bringing the animals from pasture to the handling area. Also, considering the fact that the animals used in our experiment were raised on pasture and rarely handled, our results are in accordance with the theory that extensively maintained cattle stress responses to handling might be exaggerated when compared to those of animals accustomed to interaction with humans (Millman, 2013). Our results showing decrease of heart and respiratory rates from before branding until 55 min after branding corroborate this idea and indicate that there was an effect of habituation to handling.

Values of escape attempts and tail wagging did not show any differences between groups and do not seem to be good indicators of acute pain in the conditions studied. Tail flicks and escape behaviours have already been successfully used as reliable indicators of pain for cattle branded with hot iron, normally presenting increased responses to the painful stimuli, as is shown in the results of an experiment carried out on a controlled force squeeze chute in Canada (Schwarzkopf-Genswein et al., 1997). Similarly, in an experiment measuring behavioural and physiological effects of freeze and hot iron branding on crossbred cattle in the United States, branded animals showed increased escape-avoidance reactions when compared to sham branded companions (Lay et al., 1992); however, calves observed in the same experiment were restrained but not squeezed in the chute. This was different from our situation, where animals had to be squeezed due to their size and the need for safe manipulation. The force used to restrain the animals was not measured or controlled in the present experiment, but it might have affected the freedom of animals to perform tail wagging and escape behaviours, perhaps explaining the unexpected similarity of results between the two groups.

Our results show that there was a significant effect of pain on vocal responses of cattle during branding. Although some authors suggest that vocal responses in cattle are not a robust measure of pain because of the stoic nature of cattle, which rarely vocalize (Millman, 2013), other studies have shown the scientific value of vocalizations in the assessment of cattle welfare, indicating that the acoustic structure of calls may carry different “meanings” and represent responses to states like rage, fear or pain (Watts & Stookey, 2000). In a work of Grandin (1998), the author suggests that vocalization scoring could be used as a practical and objective indicator of animal welfare in cattle slaughter plants. On Grandin’s study, aversive stimuli including painful electric prodding and missed captive bolt stuns were associated with 98.2% of vocalizations. Similarly, our results show that HB animals presented a higher proportion of individuals vocalizing more frequently and more promptly after branding than the animals on the group that went through the sham procedure (Fig. 3). These results are also in agreement with results found in an experiment where a greater proportion of calves (58/95) vocalized during hot iron branding, when compared to animals that went to the sham procedure (7/94) (Watts & Stookey, 1999). The difference of proportion of animals vocalizing in the two groups of our study indicate that the pain of branding was perceived by the animals as a more aversive stimulus than just the restraining imposed to all individuals.

All facial expressions analysed during our study presented a higher proportion of activation in HB animals, suggesting that there is an association between the display of these facial features and the pain elicited by hot iron branding. The activation of these facial expressions has already been associated with painful procedures in studies with other species. In the works of Langford et al. (2010) with laboratory mice and Keating et al. (2012) with rabbits, both backwards ear positioning and dilated nostrils have been associated with a painful stimulus. On the development of a horse grimace scale of pain (Dalla Costa et al., 2014), researchers also associated these facial expressions to pain induced by surgical castration. Our results show that all five facial expressions indicated as potential pain indicators in cattle on a pilot study (Müller et al., 2014) have indeed presented a high association with a painful stimulus and may be used in pain assessment protocols for cattle.

Some of the facial expressions described in our study have also been associated with pain in humans (Prkachin, 1992), confirming the hypothesis proposed by Darwin (1872) that facial expressions are evolutionarily conserved. The communication of pain through facial expressions is evolutionarily interesting for raising survival changes by inducing empathy in other individuals (Williams, 2002). Since humans are equipped with specialized neural apparatus and are able to recognize and process facial expressions in different species (Waller & Micheletta, 2013), training for pain assessment in animals' faces should not be complicated and it could represent a benefit for ourselves and for the animals (Flecknell, 2010).

Results also show that there was a more complex combination of facial expressions in HB animals, when compared to those that experienced the sham procedure. The activation of more than one facial feature as a result of pain was expected. Generally, a facial expression displayed in response to a certain stimulus is composed of several facial features, forming a complex grimace that is specific to the emotion experienced by the individual (Ekman et al., 1980). The higher complexity of combination of different facial features might indicate a higher severity of pain experienced by the animals (Ekman & Friesen, 1976), but such effect should be further studied.

Overall, results of facial expression were more precise in determining the painful status of cattle during branding than the other physiological and behavioural indicators analysed. Only vocalizations have shown equivalent results, suggesting that HB animals were the ones who actually experienced pain from branding. Despite the possible factors influencing the results, such as the restraining force applied and the physiological ceiling effect discussed earlier, results show that vocalization and facial expressions are more suitable for cattle pain assessment in non-laboratorial environments than cortisol, heart and respiratory rates, escape attempts and tail wagging. Additionally, although physiological measures may be useful in experimental and laboratory situations, the technical requirements make them less useful for on-farm assessment (Weary et al., 2006).

Our results show that animals in pain display a complex combination of behaviours, suggesting that an integrated measurement of different behaviours would contribute to a better pain diagnosis. However, responses to pain should be interpreted carefully because pain signs may vary between species, type of insult and stage of development (Sneddon et al., 2014). The contextualization and interpretation of multiple responses as one combined indicator may be a more accurate measure of the true state of the animal than any single indicator (Theurer et al., 2013).

3.5 CONCLUSION

Measures of cortisol, heart and respiratory rates, escape attempts and tail wagging did not seem informative about the pain status of cattle during branding and therefore are not reliable indicators for acute pain assessment; this may be related to on-farm and experimental settings, and thus warrants further research. Our results suggest that vocalization and facial expressions constitute accurate and practical indicators of pain. The integration of these parameters with other established indicators may collaborate to the improvement of on-farm pain assessment protocols for cattle.

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4. PERCEPTION OF BEEF CATTLE PRODUCERS REGARDING HOT IRON BRANDING AND ITS CONSEQUENCES TO ANIMAL WELFARE

RESUMO

O objetivo deste trabalho foi estudar a percepção de produtores de gado de corte sobre a marcação a ferro quente e suas consequências para o bem-estar animal. Dezessete produtores de gado de corte responderam um questionário sobre sua percepção acerca da identificação de bovinos e sobre aspectos de bem-estar animal. Os resultados mostraram consenso sobre a importância da identificação dos animais em suas propriedades. A maioria dos produtores (12/17) usa o ferro quente como principal método de identificação do gado e acredita que esta seja uma prática eficiente (11/17). Considerando custos e praticidade, 10/17 produtores acreditam que existem outros métodos de identificação viáveis para utilização em suas fazendas, sendo o brinco e o microchip as alternativas mais mencionadas. Os produtores afirmaram considerar os animais seres sencientes (16/17) e capazes de experimentar dor (17/17). Em uma escala de 1-5, os escores atribuídos pelos produtores à capacidade de sentir dor em diferentes espécies foram mais altos para bebês humanos (5.0, variando de 3.0 a 5.0), quando comparados com os escores dados a borboletas (2.0, 1.0-5.0) e a baratas (1.0, 1.0-5.0), mas similares aos escores atribuídos a bovinos e outros animais de produção. O escore mediano atribuído à dor que o bovino sente ao ser marcado com ferro quente foi 4.0, variando de 2.0 a 5.0. A opinião expressa pelos produtores indica que o reconhecimento da senciência animal e da dor experimentada pelos animais que estão sob seus cuidados não é um obstáculo na direção de mudanças nos procedimentos de identificação. Esforços futuros devem ser concentrados em refinar e desenvolver novos métodos que sejam acessíveis e efetivos, motivando os produtores a realizar procedimentos que respeitem a qualidade de vida dos seus animais.

Palavras-chave: Bem-estar animal, opinião, pecuaristas, identificação, gado

ABSTRACT

The aim of this study was to study the perception of beef cattle producers about hot iron branding and its consequences to animal welfare. Seventeen beef cattle producers answered a questionnaire about their perspective on cattle identification methods and animal welfare aspects. Results showed that there is a consensus among farmers that the identification of animals at their farms is an important practice. The majority of farmers (12/17) use hot iron branding as the main method of identification of cattle and most farmers (11/17) believe it is an efficient method. Considering costs and applicability, 10/17 farmers believe there are other methods of identification that would be viable for utilization at their farms; ear tagging (7/17) and microchipping (3/17) were the most mentioned alternatives. Farmers affirmed believing that animals are sentient beings (16/17) and capable of experiencing pain (17/17). On a scale from 1-5, scores attributed to pain experienced capabilities of different species were higher for human babies (5.0, ranging from 3.0 to 5.0) when compared to scores given to butterflies (2.0, 1.0-5.0) and cockroaches (1.0, 1.0-50), but similar to scores given to cattle and other farm animals. The median score attributed to the pain experienced by cattle during branding with a hot iron was 4.0, ranging from 2.0 to 5.0. The opinion expressed by producers indicates that the recognition of animal sentience and the pain experienced by animals is not an impediment to changes on identification procedures. Future efforts should focus on refining and developing new methods that are effective and inexpensive, motivating producers to use procedures that respect the quality of life of their animals.

Key words: Animal welfare, opinion, farmers, identification, bovine

4.1 INTRODUCTION

Although probing the emotional lives of non-human animals is still considered a big challenge for science, a wide variety of species show physiological and behavioral signs indicating that they experience pain (Sneddon et al., 2014). Recently, concerns about animal welfare have focused largely on the pain and distress animals may experience as a result of common practices held on farms (Von Keyserlingk et al., 2009). Of many other affective states that animals experience, pain is the most emotive of public concerns about animal welfare (Weary et al., 2006).

In beef cattle farms, animals are often submitted to management practices that are considered important to maintain control and productivity but have a high cost to the quality of life of cattle. In this balance of values, the priorities of the animals are commonly overlooked. Studies show pain related responses to practices such as dehorning (Stafford & Mellor, 2011), castration (Coetzee, 2013), tail docking (American Veterinary Medical Association, 2012), and branding (Schwarzkopf-Genswein et al., 1997), many of which are carried without proper pain control (Schwartzkopf-Genswein et al., 2012).

Of all painful practices performed at beef cattle farms, hot iron branding is of special interest. It is still common practice in cattle farms all over the world, despite all the scientific information indicating its aversive effects on animals and also its lack of efficiency on actually identifying animals (Lindegaard & Andersen, 2012). Branding is required by various governments, for example, to facilitate the export of cattle from Canada to the United States (Schwartzkopf-Genswein et al., 2012). In Brazil, all cattle vaccinated for brucellosis are required by law to be branded with a “V” shaped hot iron on the left side of the face, with no recommendation about pain control (Brasil - Ministério de Agricultura Pecuária e Abastecimento, 2006). Hot iron branding impacts animal welfare negatively in at least three different aspects: stress due to restraining the animal before and during the procedure, immediate pain during branding, and pain in the hours following the procedure (Rushen et al., 2009). Cattle responses to branding include increases in heart rate and plasma cortisol, escape avoidance reactions, tail flicking, kicking, and vocalization, all indicative of discomfort and pain. For example, in a study conducted in Canada, cattle being branded with hot iron showed significantly greater frequencies of tail flicks, kicks, falls in the chute, and vocalizations than animals experiencing a sham branding procedure (Schwarzkopf-Genswein et al., 1997). Recently, on a recent work developed by our research group, cattle being branded with hot iron vocalized more frequently and displayed specific facial expressions associated with pain in a higher proportion than animals sham branded (Müller et al., 2014).

The availability of other less painful methods for individual identification of cattle leads to doubts about the actual need of hot iron branding. For example, freeze branding consistently appears to cause less pain to cattle than traditional hot iron branding (Lay et al., 1991; Schwarzkopf-Genswein et al., 1997). Individual identification can also be achieved by other relatively less invasive practices such as ear tagging, tattooing, and microchip implantation. In a study with horses, microchip implantation resulted in less pronounced pain reactions than hot iron branding. In this case, branding, but not microchip implantation, caused necrotizing burn wounds and generalized increased superficial body temperature, which are indicative of significant tissue damage (Erber et al., 2012). A change on identification regimes at farms from hot iron branding to other methods could represent the end of a practice that causes needless pain and discomfort to the animals in our care and which is also outdated and at odds with legislative advances and public opinion (Lindegaard & Andersen, 2012).

The implementation of such changes, however, requires all stakeholders, especially farmers, to designate their perspective and address possible restrains (Weary et al., 2006). Although there is a general agreement about the effects of pain on animal welfare, farmers may perceive little opportunity for attenuating these problems without serious economic drawbacks, leading to a conflict between interests and values (Millman, 2013). In order for pain mitigation strategies to be actively adopted, they must be effective for the animals, but also available and in harmony with public concern and farmer expectations (Von Keyserlingk & Hötzel, 2015; Schwartzkopf-Genswein et al., 2012).

The aim of this study was to identify the perception of beef cattle producers about hot iron branding and its consequences to animal welfare, thus collaborating to the discussion about the methods of cattle identification and future perspectives on the adoption of less invasive and less painful practices.

4.2 MATERIAL AND METHODS

This experiment was approved by the Research Ethics Committee at the Health Science Sector of the Universidade Federal do Paraná (Federal University of Paraná, Brazil) during session on December 11, 2014, and is registered under the protocol number 909402 (Anexo 2).

A questionnaire was developed to investigate producer knowledge and perspectives about identification methods for cattle and his/her opinion on animal welfare aspects. The full questionnaire contained 14 objective and open questions, of which five were demographic inquires, five were related to cattle identification methods, and four regarded animal welfare issues (Tab. 4).

TABLE 4. NON-DEMOGRAPHIC QUESTIONS AND POSSIBLE ANSWERS PRESENT ON THE QUESTIONNAIRE GIVEN TO BEEF CATTLE FARMERS IN THE STATE OF PARANÁ, SOUTHERN BRAZIL, 2015.

Questions	Possible answers
Question 6 – Do you believe animal identification is an important practice at your farm?	( )Yes ( )No
Question 7 – Is hot iron branding the standard procedure for identification of cattle in your farm?	( )Yes ( )No
Question 7a) If yes, for how long have you been using hot iron branding?	Open answer
Question 7b) If not, which other identification method do you use at your farm?	Open answer
Question 8 – Do you believe hot iron branding is an efficient method for identification of cattle?	( )Yes ( )No
Question 9 – Do you know any other methods for identification of cattle? Which other methods do you know?	Open answer
Question 10 – Considering costs and applicability, do you believe other methods of identification are viable for utilization at your farm?	( )Yes ( )No
Question 10a) If yes, which?	Open answer
Question 10b) If not, why?	Open answer
Question 11 – Do you believe animals are sentient beings, meaning they are capable of experiencing feelings?	( )Yes ( )No

Question 12 – Do you believe animals are capable of experiencing pain?	( )Yes ( )No
Question 13 – In a scale from 1 to 5, where 1=none and 5=maximum imaginable, what is the capability of each of the following animals of experiencing pain: Pigeon, Butterfly, Human baby, Rat, Dog, Chicken, Fish, Sheep, Cattle, Cockroach, Wolf	( )1 ( )2 ( )3 ( )4 ( )5 ( )I don't know
Question 14 – In a scale from 1 to 5, where 1=none and 5=maximum imaginable, how much pain do you believe cattle experience during branding with a hot iron?	( )1 ( )2 ( )3 ( )4 ( )5 ( )I don't know

In order to obtain contact details of beef cattle producers in the State of Paraná, a total of nine institutions related to the beef cattle industry were contacted, including governmental organizations, producer associations, and private companies. At first contact, institutions were asked about their interest on participating on the project and, in the case of a positive answer, registered producer contact information was required for direct communication via telephone. All institutions received a short description of the project, and the full questionnaire with a copy of the approval letter by the Ethics Committee attached.

When farmer contact details were provided, they were reached via phone calls, provided with a brief explanation about the project and asked about their interest on contributing to the research. If they were willing to participate, producers were instructed to answer the questionnaire, which took them about five minutes to complete.

All objective and open answers were compiled and simple descriptive analysis was performed. Effects of species on the attributed pain capability scores given to animals by farmers, as well as effects of demographic status on the use of hot iron branding as standard procedure for identification of cattle, on general scores attributed by farmers to animals' capability of experiencing pain, and on the score given by producers to the perceived pain intensity experienced by cattle during hot iron branding were tested using the Kruskal-Wallis test followed by the Dunn's test for classification of results. All statistical analyses were performed using the statistical software BioEstat 5.0 (Instituto Mamirauá, 2007).

4.3 RESULTS

Only one of all nine institutions agreed to participate in the project. Five institutions denied disclosing producer information after analyzing the description of the project and the questionnaire. Most negative answers were justified upon privacy policies and protection of producer information. The institution which agreed to participate contributed with contact details of eleven producers, which represented all farmers registered with them. Contact detail of thirteen other producers was kindly provided by one producer who demonstrated great interest on the survey. After contacting all 24 producers, a total of 17 were willing to participate and answered the questionnaire.

Demographic information about producers showed that 16 out of 17 producers interviewed were male, 11/17 were 40 years or older, and 10/17 had completed higher education. Only 3/17 producers declared that farming was their full-time occupation, while 6/17 also work as veterinarians and 8/17 had other jobs including agronomy (1/16), sales (1/16), civil engineering (1/16), earthmoving (1/16), legal advisory (1/16), and business (3/16). The most common city of residence was the State capital Curitiba, where 7/17 producers lived, while 5/17 lived in Palmeira, 1/17 in Ortigueira, 1/17 in Campo do Tenente, 1/17 in Cascavel, 1/17 in Paranavaí, and 1/17 in Campina Grande do Sul. Demographic information from interviewed farmers can be seen on Fig. 5.

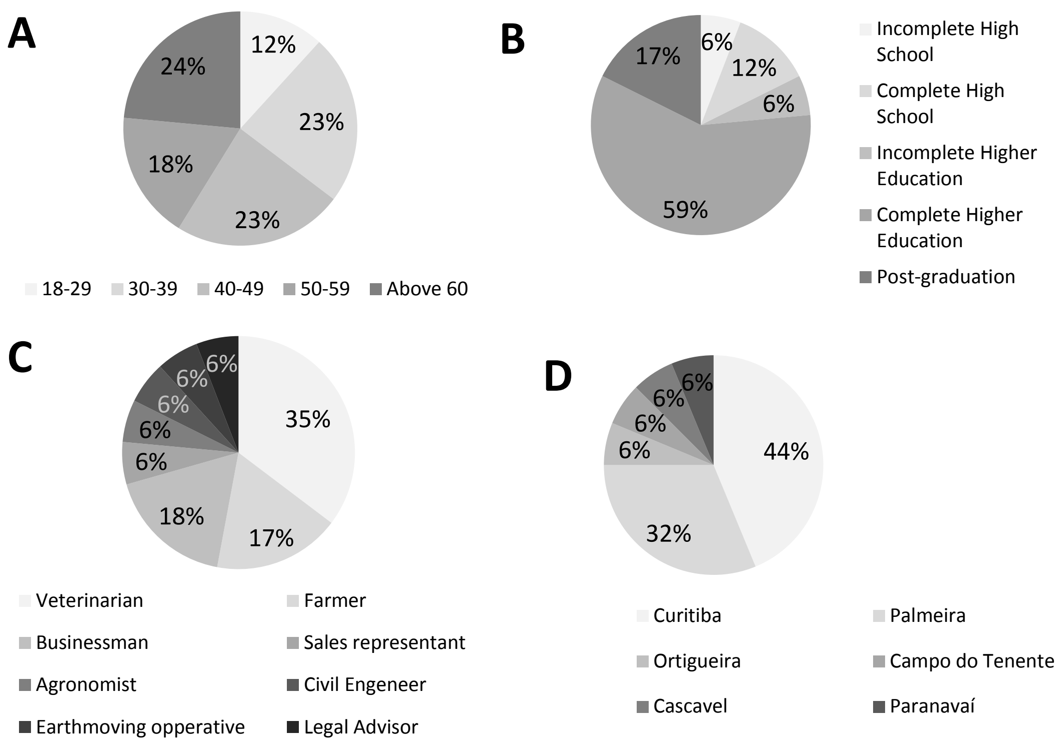

FIGURE 5. DEMOGRAPHIC INFORMATION OF 17 BEEF CATTLE PRODUCERS INTERVIEWED IN THE STATE OF PARANÁ, 2015. GRAPHICS REPRESENT PERCENTAGES OF AGE GROUP (A), EDUCATION (B), OCCUPATION (C), AND CITY OF RESIDENCE (D).

Producers were unanimous (17/17) when declaring that the identification of animals in their farms is an important practice. When asked about the standard method of identification used by them, 12/17 stated that hot iron branding is the method of choice, and 5/17 stated that they use ear tagging . Producers who use hot iron branding reported that they have been using this method for 25 ± 13 years.

The majority of the producers (11/17) believe that hot iron branding is an efficient method for identification of cattle. All producers stated that they know at least one other method of identification, micro chipping and ear tagging being the most popular with 11/17 producers making reference to these methods, followed by ear tattooing (cited by 9/17 producers), ear clipping (3/17), freeze branding (3/17), and intra-ruminal transponder (1/17). Considering costs and applicability, 10/17 producers declared some alternative methods are viable for utilization on their farms. The most quoted viable method of choice was ear tagging (cited by 7/17 producers), followed by micro chipping (3/17), and ear tattooing (2/17). The main reason why producers wouldn't consider using another method for identification was the costs involved, mentioned by all farmers who answered "No" to question 10 (7/17 producers).

All but one farmer (16/17) believe animals are sentient beings and there was a common agreement (17/17) that animals are capable of experiencing pain. When asked about the capability of experiencing pain in different species, median scores were 4.0 (minimum 1 and maximum 5) for pigeons; 2.0 (1-5) for butterflies; 5.0 (3-5) for human babies; 4.0, (1-5) for rats; 5.0 (2-5) for dogs; 3.5 (1-5) for chickens; 2.5 (1-5) for fish; 5.0 (2-5) for sheep; 4.0 (3-5) for cattle; 1.0 (1-5) for cockroaches; and 4.5 (2-5) for wolves. There was an effect of species on the attributed pain capability score given to animals by farmers (*P*<0.01). The human baby median score was statistically higher than median scores of the butterfly and cockroaches (*P*<0.05), dog and cattle median scores were similar to human baby mean score but also higher than cockroach mean score (*P*<0.05) (Fig. 6). The median score given to the pain producers believe cattle experience during branding was 4.0, ranging from a minimum of 2 and a maximum of 5.

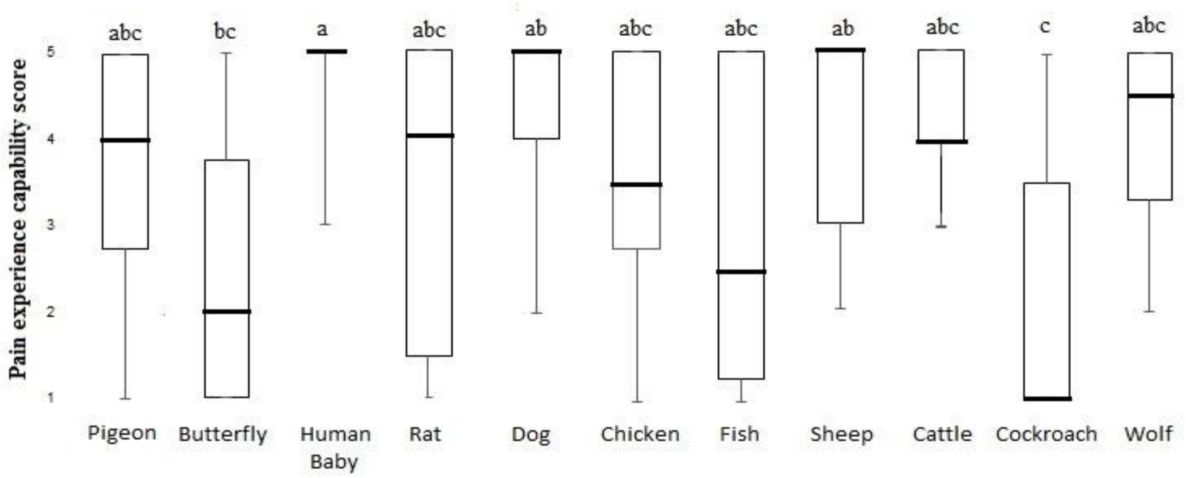

FIGURE 6. MEDIAN PERCEIVED PAIN EXPERIENCE CAPABILITY SCORES GIVEN TO DIFFERENT SPECIES BY 17 BEEF CATTLE FARMERS INTERVIEWED IN THE STATE OF PARANÁ, SOUTHERN BRAZIL, 2015. MEDIAN BOX PLOTS ACCOMPANIED BY DIFFERENT LETTERS INDICATE STATISTICAL DIFFERENCES BETWEEN SPECIES GIVEN SCORES (P<0.05).

There was no association between age group and use of hot iron branding as standard procedure for identification of cattle (*P*=0.72). There was an effect of age group on general scores attributed by farmers to animal capability of experiencing pain (*P*<0.05). Producers in the age group of 50-59 years old gave similar scores for pain capability than producers in the age groups 18-29, 40-49, and 60 years or older, but constantly gave higher scores than producers in the age group 30-39 years old (Fig. 7). There was no effect of age group on the score given by producers to the perceived pain intensity experienced by cattle during hot iron branding (*P*=0.50).

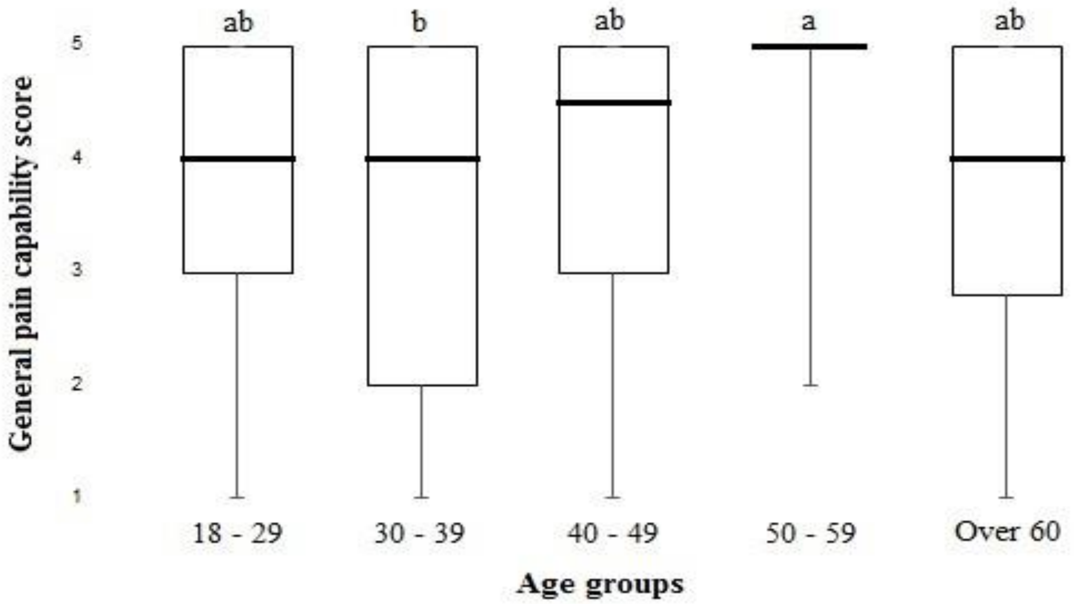

FIGURE 7. MEDIAN SCORES GIVEN BY 17 BEEF CATTLE FARMERS TO THE PERCEIVED PAIN EXPERIENCE CAPABILITY OF DIFFERENT SPECIES DURING AN INTERVIEW REALIZED IN THE STATE OF PARANÁ, SOUTHERN BRAZIL, 2015. MEDIAN BOX PLOTS ACCOMPANIED BY DIFFERENT LETTERS INDICATE STATISTICAL DIFFERENCES BETWEEN AGE GROUPS (P<0.05).

Due to the high prevalence of males and producers with complete higher education, data was not sufficiently homogeneous to test for any possible effects of gender and education on the answers given.

4.4 DISCUSSION

The number of positive responses to participate in the survey was very low considering the official number of beef cattle producers in the State of Paraná, with its 55,873 registered farms (Mezzadri, 2013). Low participation rates are common in survey research, and responses to questionnaires are low even when there are monetary incentives (Deutskens et al., 2004). On a recent survey discussing difficulties encountered by beef cattle producers in adopting a traceability system in the State of Minas Gerais, Brazil, the number of participating producers was also low, with a total of 20 respondents (Lopes et al., 2012). Similarly, on a survey describing farmer perceptions of animal welfare in the Netherlands, a total of 15 farmers were interviewed (Te Velde et al., 2002). Confidentiality restrains in governmental institutions and a possible indisposition with the survey topics on private institutions may explain the difficulty in reaching producers. The development of a cooperative research, in partnership with those institutions and addressing shared issues, should be more effective in that matter. Even though our results are not representative of the population of producers in the State of Paraná, collected data may give relevant preliminary information to address issues related to animal identification and animal welfare from the farmer perspective. The low variability of producers perspectives on questions related to the importance of cattle identification, to animal sentience and capability of experiencing pain suggests that in these issues results may have some predictive value. Interpretation of contextualized data, no matter how limited it is, might contribute significantly to the establishment of new references, important to guide future research (Veronese & Guareschi, 2006).

There has been a significant advance on global standards and requirements for cattle identification (Schroeder & Tonsor, 2012). Identification of cattle is of paramount importance to ensure control of productivity parameters, as well as to ensure differentiation between farm herds and to guarantee disease control and traceability. Producers interviewed in our survey seem to understand these issues and consider identification as an essential practice. The unanimity about the importance of identification showed by producers indicates that there is a demand for reliable methods of identification. This demand is also described in other countries with traditional beef cattle production such as Australia (Petherick, 2005), Canada (Stanford et al., 2001), and the United States (Schroeder & Tonsor, 2012).

The main methods of cattle identification used by the surveyed farmers are hot iron branding and ear tagging. This is in accordance with a study conducted in Brazil, where the percentage of producers that use ear tags, hot iron branding, or both methods combined summed up to 80% (Lopes et al., 2012). Producers who declared the use of hot iron branding have been using this method at their farms for more than two decades, suggesting that the adoption of this practice is not recent, but could be interpreted as a form of “tradition”. At newer farms, producers might be prone to use additional identification techniques that seemed impossible or expensive a few years ago, but that are now available and more affordable (Stookey & Watts, 2004). Recent international changes on traceability policies might be influencing producers to use methods with trace-back capabilities, which may contribute to the obsolescence of hot iron branding (Schroeder & Tonsor, 2012).

The majority of interviewed producers declared they believe hot iron branding is an effective method for identification. Superiority of the hot iron branding over other methods is often defended by farmers that discuss that branding scars can be read at distance (Lindegaard & Andersen, 2012). Contrary to this assertion, results from a study with horses show that hot iron branding does not allow reliable identification of animals due to hair growth around the branding mark and one of the digits often being ineligible (Aurich et al., 2013). Another commonly mentioned advantage of hot iron branding is the low costs related to the method (Schwarzkopf-Genswein et al., 1997). Indeed, cost was the most common answer, given by all producers in our study when asked about the reasons why they wouldn't consider other methods viable for application at their farms. Even though producers know a number of alternative methods, these do not seem to be economically attractive for widespread adoption. However, the aspect of costs related to management procedures in farms is a complex matter. Stressful practices are known to have significant effects on productivity indexes of farm animals, representing an indirect cost related to such procedures (Broom, 1997). A detailed study of the costs involved with different methods of identification of cattle should be helpful at clarifying major influences and determining the real economic aspects of each practice.

There was a high percentage of producers in our study stating that there are alternative practices to hot iron branding which are potentially viable for use. High prevalence of producers using ear tagging and micro chipping as alternative methods for identification might be an indicative of the route to be taken. Although both methods present drawbacks that must be addressed, such as relative high costs, low time persistence and difficulty of reading (Johnston & Edwards, 1996; Petherick, 2005; Stanford et al., 2001), their potential in reducing animal suffering and their efficiency as a means of ID for cattle (Løken et al., 2011) appear to be in accordance to worldwide trends in animal traceability and public concerns about animal welfare (Lindegaard & Andersen, 2012; Schroeder & Tonsor, 2012).

Interviewed producers recognize animals are sentient beings, capable of experiencing pain. One producer, however, answered that although animals are capable of experiencing pain, they are not capable of experiencing feelings. The emotional component of pain in animals is indeed a controversial subject open to debate (Treede, 2006). However, a growing body of research on the motivational and subjective aspects of behaviors (Désiré et al., 2002) indicate that the complexity of responses to pain go beyond simple and acute detection and reflex responses and begin to demonstrate a level of behavioral complexity that would require some form of experience (Sneddon et al., 2014). Indeed, pain in animals has been recognized as an aversive sensory and emotional experience since 1997 (Molony & Kent, 1997).

Generally, farmers believe human babies possess higher ability to experience pain, but they attributed similar scores to cattle, suggesting that they agree with scientific suggestion that the animals under their care might experience pain in a similar way to humans (Sneddon et al., 2014). Similar results were obtained in a study conducted in Norway, where the majority of dairy farmers either agreed (39%) or totally agreed (31%) with the statement that animals experience physical pain as humans do (Kielland et al., 2010). Lower pain capability scores attributed to animals by producers aged between 30-39 years old suggests that younger producers are less likely to recognize pain than producers aged between 50-59 years old. This might be associated to practical knowledge or emotional maturity, yet elucidation about the real factors contributing to this effect may be better detailed on further research.

Differences in absolute scores given to sheep, cattle and chickens deserve further investigation as the husbandry and welfare of these animals might be influenced by the producer perceived impact of management practices on animal lives (Ohl & Van der Staay, 2012). Lower scores attributed to invertebrates (butterflies and cockroaches) are coherent with scientific uncertainty about the real aspects of pain in these animals (Sneddon et al., 2014)

When asked about how much pain they believe cattle feel when branded with a hot iron, most farmers attributed high scores. This result, associated to the fact that many of the interviewed farmers still use hot iron branding, confirms the scientific suggestion that although producers might recognize the pain associated to specific procedures, they do not always act to mitigate it (Millman, 2013). However, Bath (1998) suggests that changes must begin with awareness, and farmer recognition of the pain involved on hot iron branding might be considered per se as an important step towards the adoption of alternative methods. Thus, for further improvement on attitudes towards adequate pain management in animals, it is important that new, robust and practically useful methods for pain diagnosis be developed and that producers learn to identify painful procedures conducted in their farms (Flecknell & Roughan, 2004; Millman, 2013); producers should also be provided with information about adequate pain management methods (Hawkins, 2002; Schwartzkopf-Genswein et al., 2012) and feel motivated to enhance the welfare of the animals under their care (Weary et al., 2006).

4.5 CONCLUSION

Recent international concerns about the identification of cattle seem to be also shared by producers. Although hot iron branding is a widespread method for identification of animals, it appears that alternative practices are getting more popular among producers, probably encouraged by new trends in traceability policies and public opinion. Producer awareness about animal sentience and the pain experienced by the animals under their care might indicate a step towards change on identification procedures. In terms of animal welfare, future efforts should focus on refining and developing new methods that are effective and inexpensive, motivating producers to use procedures that respect the quality of life of their animals.

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5. CONSIDERAÇÕES FINAIS

O avanço nos métodos de diagnóstico da dor e na adoção de práticas de manejo mais compassivas interfere diretamente na vida dos animais que estão sob nossos cuidados. As conclusões apresentadas neste trabalho, se aplicadas nos sistemas produtivos, podem gerar mudanças importantes e levar a uma melhoria direta no grau de bem-estar de bovinos de corte.

A expressão facial como método de diagnóstico de dor pode se tornar uma ferramenta útil em situações de campo, facilitando o reconhecimento, a prevenção e o controle da dor em ambientes onde não haja disponibilidade de equipamentos complexos ou tempo para análises mais demoradas. Ainda assim, as estratégias de treinamento para utilização deste método necessitam ser definidas e a variação de diagnóstico entre avaliadores deve ser estudada. Estudos futuros sobre as associações entre diferentes indicadores nas respostas à dor podem contribuir para um diagnóstico mais preciso. De forma mais imediata, o desenvolvimento de materiais informativos sobre o uso integrado dos indicadores apresentados nesta dissertação parece ser uma forma interessante de aplicação prática dos conhecimentos gerados.

A tradição e a percepção de baixos custos relativos à marcação a ferro quente parecem ser as causas da continuidade de utilização deste método. Entretanto, o cenário de percepção dos produtores demonstra que eles parecem estar preparados para a adoção de alternativas. A ponderação sobre os reais custos monetários e morais da marcação a ferro quente pode representar o ponto de inflexão para a tomada de decisão por novos métodos.

APÊNDICES

APÊNDICE 1

Unidades Faciais de Ação Associadas à Dor em Bovinos de Corte

Bruno R. Müller, Janaina Hammerschmidt, Claudia S. Feldens, Carla F.M. Molento
LABEA
Laboratório de Bem-estar Animal - Universidade Federal do Paraná

INTRODUÇÃO

Apesar da ciência de expressões faciais de dor em humanos estar bastante avançada, ela não tem sido muito explorada em animais. O estudo da expressão facial como indicador de dor pode representar um avanço significativo no reconhecimento e tratamento da dor em espécies ainda não estudadas. O objetivo deste estudo foi investigar se unidades de ação (UA) faciais específicas, previamente relacionadas à expressão facial de dor em humanos e algumas espécies de animais, também são ativadas em bovinos de corte durante estimulação aguda de dor.

MATERIAL E MÉTODOS

A ativação das UA foi analisada comparativamente por meio de fotos de 35 bovinos de corte em momentos antes e durante a marcação com ferro quente, representando situações de ausência e presença de dor, respectivamente. Os animais observados foram 17 fêmeas e 18 machos de dois genótipos diferentes: Nelore e cruzados (1/2 Nelore, 1/4 Bosmara, 1/8 Red Angus e 1/8 Aberdeen Angus).

RESULTADOS E DISCUSSÃO

Os resultados mostraram que não houve diferença significativa de ativação das UA entre machos e fêmeas e, quando comparados aos animais da raça nelore, os bovinos cruzados apresentaram maior frequência de abertura de boca. A ativação das UA orelhas para trás, narina dilatada, abertura de boca e elevação medial e lateral das sobrancelhas apresentou significativa associação com a presença do estímulo doloroso, aqui representado pela marcação a ferro quente (Tabela 1).

CONCLUSÕES

As UA orelhas para trás, narina dilatada, abertura de boca e elevação medial e lateral das sobrancelhas são ativadas durante a marcação a ferro quente em bovinos de corte (Figura 1) e, portanto, devem ser consideradas no desenvolvimento de futuros métodos de diagnóstico de dor que utilizem a expressão facial como indicador para esta espécie.

Tabela 1 – Número total de animais observados e frequência de ativação das UA: orelhas para trás (OT), narina dilatada (ND), abertura de boca (AB) e elevação medial (EM) e lateral (EL) da sobrancelha em bovinos submetidos a ausência (A) e presença (P) de estímulo doloroso.

Ativação das UA	OT	ND	AB	EM	EL
A e P	4	0	0	9	3
Somente A	0	1	0	2	0
Somente P	8	20	17	14	16
Nenhum	3	4	13	10	14
Total de animais	**15**	**25**	**30**	**35**	**33**

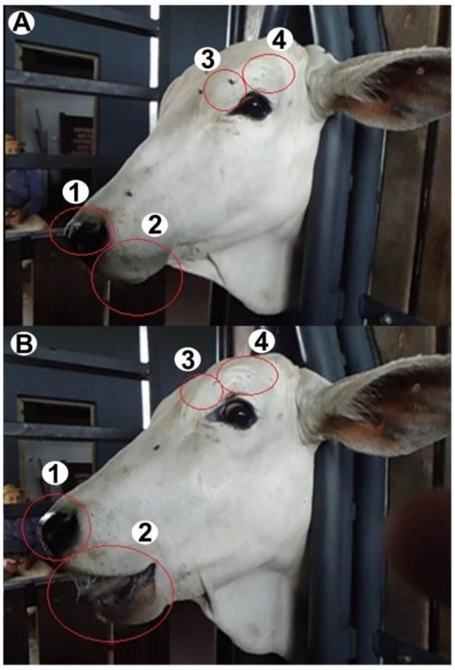

Figura 1 – Unidades de ação: narina dilatada (1), abertura de boca (2) e elevação medial (3) e lateral (4) da sobrancelha, desativadas (A) e ativadas (B) no mesmo animal em momentos antes e durante a marcação a ferro quente, respectivamente.

REFERÊNCIAS

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Ekman, P., 1993. Facial expression and emotion. Am. Psychol. 48, 384–92.
Dalla Costa, E., Minero, M., Lebelt, D., Stucke, D., Canali, E., Leach, M.C., 2014. Development of the Horse Grimace Scale (HGS) as a Pain Assessment Tool in Horses Undergoing Routine Castration. PLoS One 9, e92281.

UFPR 100 Anos 1912-2012
Fazenda Arca de Noé
BECKHAUSER Manejo Racional e Produtivo
CAPES

ANEXOS

ANEXO 1

APROVAÇÃO NA COMISSÃO DE ÉTICA NO USO DE ANIMAIS DO SETOR DE CIÊNCIAS AGRÁRIAS DA UNIVERSIDADE FEDERAL DO PARANÁ

UFPR

Universidade Federal do Paraná
Setor de Ciências Agrárias
Comissão de Ética no Uso de Animais – CEUA SCA

CERTIFICADO

Certificamos que o protocolo no. 074/2013, referente ao projeto "A expressão facial como indicador de dor em bovinos", sob a responsabilidade de Bruno Roberto Müller, na forma em que foi apresentado (uso de 60 bovinos), foi aprovado pela Comissão de Ética no Uso de Animais do Setor de Ciências Agrárias, em reunião realizada dia 16 de dezembro de 2013.

CERTIFICATE

We certify that the protocol number 074/2013, regarding the project "Facial expression as pain indicator in cattle", under Bruno Roberto Müller's supervision, in the terms it was presented (use of 60 steers), was approved by the Animal Use Ethics Committee of the Agricultural Sciences Campus of the Universidade Federal do Paraná (Federal University of the State of Paraná, Brazil) during session on December 16, 2013.

Curitiba, 17 de dezembro de 2013.

Patrick Schmidt
Presidente

Ricardo Guilherme D´Otaviano de Castro Vilani
Vice-Presidente

Comissão de Ética no Uso de Animais
Setor de Ciências Agrárias
Universidade Federal do Paraná.

ANEXO 2

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO E APROVAÇÃO NO COMITÊ DE ÉTICA EM PESQUISA COM HUMANOS DO SETOR DE CIÊNCIAS DA SAÚDE DA UNIVERSIDADE FEDERAL DO PARANÁ

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Nós, Carla Forte Maiolino Molento e Bruno Roberto Müller, pesquisadores da Universidade Federal do Paraná, estamos convidando os produtores de bovinos do Estado do Paraná a participar do projeto "Percepção e atitudes humanas sobre a senciência animal e questões relacionadas ao bem-estar animal", subprojeto "Percepção de produtores de bovinos de corte acerca da prática de marcação a ferro quente e suas consequências para o bem-estar animal", por meio da aplicação de um questionário. A presente pesquisa justifica-se em função da crescente preocupação com o bem-estar dos animais e a necessidade do levantamento de dados a respeito da percepção dos bovinocultores acerca da realização da marcação a ferro quente em bovinos.

a) O objetivo desta pesquisa é criar subsídios para uma discussão dos métodos de identificação de bovinos de corte e para a reformulação das recomendações acerca da identificação de animais vacinados contra brucelose..

b) Caso você participe da pesquisa, será necessário responder ao questionário apresentado, contendo perguntas abertas e fechadas.

c) Alguns riscos relacionados ao estudo podem ser: abertura de informação de dados demográficos (idade, sexo), os quais serão confidenciais, anônimos, visíveis apenas pelos responsáveis por esta pesquisa; caso haja qualquer constrangimento por acreditar que está correndo risco de ser julgado, o participante poderá deixar de responder às perguntas a qualquer momento.

d) Os benefícios esperados com essa pesquisa são: a contribuição para o avanço científico na área de bem-estar animal, bem como da relação ser humano-animal.

e) As pesquisadoras Dra. Carla Forte Maiolino Molento, médica veterinária, professora associada da Universidade Federal do Paraná-UFPR, vinculada ao Programa de Pós-Graduação em Ciências Veterinárias da Universidade Federal do Paraná - UFPR e coordenadora do Laboratório de Bem-estar animal (LABEA) (Email: carlamolento@yahoo.com; Telefone fixo: 41 3350-5788; Celular: 41 9931-6302) e Bruno Roberto Müller, zootecnista e aluno de mestrado do Programa de Pós-Graduação em Ciências Veterinárias da Universidade Federal do Paraná - UFPR (Email: brunormuller@yahoo.com.br; Telefone fixo: 41 3350-5788; Celular: 41 9107-0405), responsáveis por este estudo, poderão ser contatadas por meio do seguinte endereço: Laboratório de Bem-estar Animal (LABEA), Universidade Federal do Paraná (UFPR), Setor de Ciências Agrárias, Departamento de Zootecnia, Rua dos Funcionários, 1540, CEP: 80035-050, Curitiba, PR, das 07h30min às 17h30min, nos telefones supracitados, para esclarecer eventuais dúvidas e fornecer-lhe as informações que queira, antes, durante ou depois de encerrado o estudo.

k) A sua participação neste estudo é voluntária e se você não quiser mais fazer parte da pesquisa poderá desistir a qualquer momento e solicitar que lhe devolvam o termo de consentimento livre e esclarecido assinado.

l) As informações relacionadas ao estudo poderão ser conhecidas por pessoas autorizadas, como a Dra. Carla Forte Maiolino Molento e Bruno Roberto Müller, autores da pesquisa. No entanto, se qualquer informação for divulgada em relatório ou publicação, isto será feito sob forma codificada, para que a sua identidade seja preservada e mantida a confidencialidade.

Comitê de ética em Pesquisa do Setor de Ciências da Saúde da FUFPR
Rua Pe. Camargo, 280 – 2° andar – Alto da Glória – Curitiba-PR –CEP:80060-240
Tel (41)3360-7259 - e-mail: cometica.saude@ufpr.br

Aprovado pelo Comitê de Ética em Pesquisa em Seres Humanos do Setor de Ciências da Saúde/UFPR.
Parecer CEP/SD-PB nº 909402
na data de 11 . 12 . 2014

Rubricas:
Participante da Pesquisa:
Pesquisador Responsável

n) Quando os resultados forem publicados, não aparecerá seu nome, e sim um código.

Eu,__________________________________, compreendi esse termo de consentimento e a natureza e objetivo do estudo do qual concordei em participar. A explicação que recebi menciona os riscos e benefícios. Eu entendi que sou livre para interromper minha participação a qualquer momento sem justificar minha decisão e sem que esta decisão tenha qualquer prejuízo para mim.

Eu concordo voluntariamente em participar deste estudo.

____
(Assinatura do participante de pesquisa)
Local e data

Local e data:

Assinatura dos Pesquisadores:

Aprovado pelo Comitê de Ética em Pesquisa em Seres Humanos do Setor de Ciências da Saúde/UFPR.
Parecer CEP/SD-PB.nº 909 402
na data de 11 / 12 / 2014

Comitê de ética em Pesquisa do Setor de Ciências da Saúde da FUFPR
Rua Pe. Camargo, 280 – 2º andar – Alto da Glória – Curitiba-PR –CEP:80060-240
Tel (41)3360-7259 - e-mail: cometica.saude@ufpr.br

ANEXO 3

COMPROVANTE DE SUBMISSÃO DO ARTIGO REFERENTE AO SEGUNDO CAPÍTULO DESTA DISSERTAÇÃO AO PERIÓDICO ARQUIVO BRASILEIRO DE MEDICINA VETERINÁRIA E ZOOTECNIA

Arquivo Brasileiro de Medicina Veterinária e Zootecnia

SISTEMA DE PUBLICAÇÕES ON-LINE FEP MVZ Editora [English] [Portugues]

- Editar Minha conta
- Inscrição de Trabalhos (*Autores brasileiros*)
- Inscrição de Trabalhos (*Autores estrangeiros*)
- Consulta Trabalhos Enviados
- Consulta Trabalhos que faço parte (enviados por outros autores)
- Sair

Trabalhos Enviados

Imprimir

ID: **8072/2014** Data de Envio: **20/10/2014**
Título: **Expressões faciais associadas à dor em bovinos de corte**
Autores: **Bruno Roberto Müller /Janaina Hammerschmidt /Cláudia Schwarzbold Feldens /Carla Forte Maiolino Molento**
Situação: **Aguardando avaliação do comitê editorial**

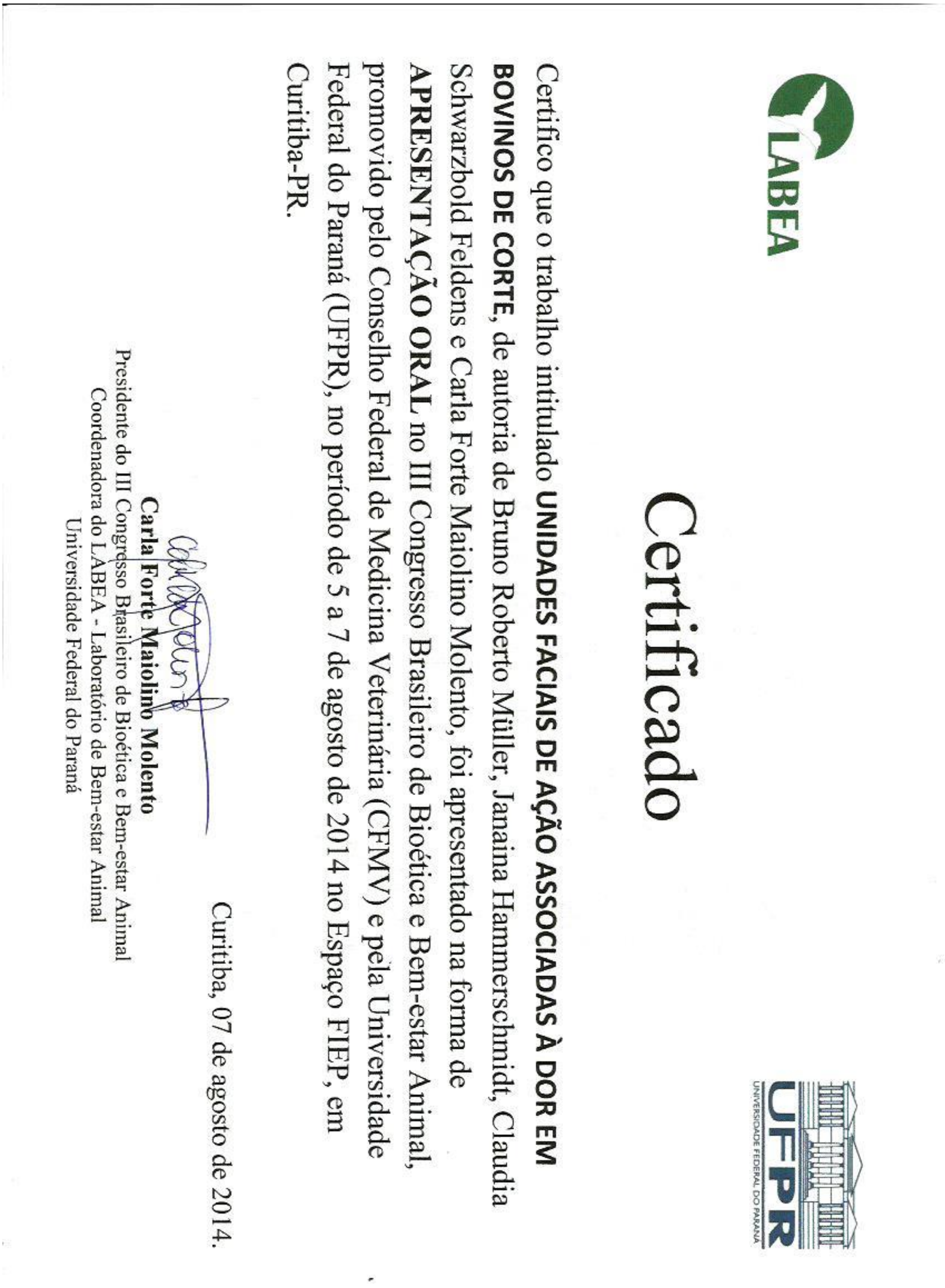
Imprimir

Apoio:

CNPq · CAPES · Ministério da Educação · Ministério da Ciência e Tecnologia · Governo Federal BRASIL · Escola de Veterinária UFMG · FAPEMIG · FEPMVZ · SBTE · PRPQ UFMG · SBMA

ANEXO 4

CERTIFICADO DE APRESENTAÇÃO ORAL NO III CONGRESSO BRASILEIRO DE BIOÉTICA E BEM-ESTAR ANIMAL



LABEA

UFPR
UNIVERSIDADE FEDERAL DO PARANÁ

Certificado

Certifico que o trabalho intitulado **UNIDADES FACIAIS DE AÇÃO ASSOCIADAS À DOR EM BOVINOS DE CORTE**, de autoria de Bruno Roberto Müller, Janaina Hammerschmidt, Claudia Schwarzbold Feldens e Carla Forte Maiolino Molento, foi apresentado na forma de **APRESENTAÇÃO ORAL** no III Congresso Brasileiro de Bioética e Bem-estar Animal, promovido pelo Conselho Federal de Medicina Veterinária (CFMV) e pela Universidade Federal do Paraná (UFPR), no período de 5 a 7 de agosto de 2014 no Espaço FIEP, em Curitiba-PR.

Curitiba, 07 de agosto de 2014.

Carla Forte Maiolino Molento
Presidente do III Congresso Brasileiro de Bioética e Bem-estar Animal
Coordenadora do LABEA - Laboratório de Bem-estar Animal
Universidade Federal do Paraná

ANEXO 5

CERTIFICADO DE APRESENTAÇÃO DE PÔSTER NO III CONGRESSO BRASILEIRO DE BIOÉTICA E BEM-ESTAR ANIMAL

Certificado

Certifico que o trabalho intitulado **UNINDADES FACIAIS DE AÇÃO ASSOCIADAS À DOR EM BOVINOS DE CORTE**, de autoria de Bruno Roberto Müller, Janaina Hammerschmidt, Claudia Schwarzbold Feldens e Carla Forte Maiolino Molento, foi apresentado na forma de **PÔSTER** no III Congresso Brasileiro de Bioética e Bem-estar Animal, promovido pelo Conselho Federal de Medicina Veterinária (CFMV) e pela Universidade Federal do Paraná (UFPR), no período de 5 a 7 de agosto de 2014 no Espaço FIEP, em Curitiba-PR.

Curitiba, 07 de agosto de 2014.

Carla Forte Maiolino Molento
Presidente do III Congresso Brasileiro de Bioética e Bem-estar Animal
Coordenadora do LABEA - Laboratório de Bem-estar Animal
Universidade Federal do Paraná

ANEXO 6

ACEITAÇÃO DE RESUMO PARA APRESENTAÇÃO NO UFAW INTERNATIONAL WELFARE SCIENCE SYMPOSIUM, ZAGREB, CROÁCIA, 2015

Animal Populations – World Resources and Animal Welfare

UFAW International Animal Welfare Science Symposium
Zagreb, Croatia 14-15th July 2015

- **Millsopp S, C Westgarth, R Barclay and M Ward** (Universities of Chester and Liverpool, UK)
  'Companion animal behaviour counselling: Are we solving animal welfare problems or human welfare problems?'
- **Müller BR, K Zeidan and CFM Molento** (Federal University of Paraná, Brazil)
  'Hot iron branding and beef cattle vocalization'
- **Mutonono - Watkiss B, E Fogelberg and E Parravani** (World Animal Protection, UK)
  'Humane and holistic dog population management'
- **Ngonyo J** (Africa Network for Animal Welfare, Kenya)
  'The central role of livestock in communities and on livelihoods with case studies from Africa'
- **Osmar-Vitalich S** (Swedish University of Agricultural Sciences, Sweden)
  'Rabies, dogs and education. A cross-sectional study on the knowledge, attitude and practice in school children in Tamil Nadu'
- **Packer RMA and HA Volk** (The Royal Veterinary College, UK)
  'Can canine inherited diseases reduce the number of laboratory animals used in research? Canine idiopathic epilepsy as a naturally occurring and humane model of idiopathic epilepsy in humans'
- **Petek M, E Cavusoglu, E Topal and IM Abdourhamane** (University of Uludag, Turkey)
  'Effects of plastic or wood slatted floor housing system on broiler welfare'
- **Radeski M and V Ilieski** (University "Ss. Cyril and Methodius" Skopje, Macedonia)
  'Age related welfare changes in dairy cows'
- **Radisavljević K, M Vučinić and A Hammond-Seaman** (University of Belgrade, Serbia; RSPCA, UK)
  'Shelter overcrowding influence on dogs health in Serbia'
- **Reaney SJ and LM Collins** (University of Lincoln, UK)
  'The mediating effect of personality on the expression and experience of pain in non-human animals'
- **Russo C, A Amici, M Farruggia and M Lo Valvo** (Universities of Pisa, Tuscia and Palermo, Italy)
  'Rearing methods of wild rabbits (*Oryctolagus cuniculus*) for reintroduction in Sicily'
- **Russo C, C Facchini, LE Della Casa, M Ferraguti and S Mattiello** (Universities of Pisa and Milan, Italy)
  'Wolf (*Canis lupus*) predation on ovine Zerasca breed in Massa- Carrara province'
- **Sa RCC, CC Burn and JCM Lewis** (The Royal Veterinary College and Wildlife Vets International, UK)
  'Surviving reintroduction: behavioural responses of captive bred Amur leopard, *Panthera pardus orientalis*, to Amur tiger, *Panthera tigris altaica*, faeces'
- **Saraiva S, A Esteves, I Oliveira and G Stilwell** (Universities of Trás-os-Montes e Alto Douro and Lisbon, Portugal)
  'Fear, physical condition and mortality as indicators of hens' welfare during the laying period'
- **Saraiva S, C Saraiva and G Stilwell** (Directorate-General of Food and Veterinary and Universities of Trás-os-Montes e Alto Douro and Lisbon, Portugal)
  'Evaluation of broiler welfare at the slaughterhouse in Portugal'
- **Schad KM and K Leus** (European Association of Zoos and Aquaria, The Netherlands; Copenhagen Zoo, Denmark)
  'Population management in zoos and aquaria'

UFAW

Science in the Service of Animal Welfare

ANEXO 7

TERMO DE COMPROMISSO DE ESTÁGIO GERADO A PARTIR DOS DADOS DE VOCALIZAÇÃO DO CAPÍTULO 3 DESTA DISSERTAÇÃO

ESTAGIO NO ÂMBITO DA UFPR

TERMO DE COMPROMISSO PARA ESTÁGIO
PARA ALUNOS DA UNIVERSIDADE FEDERAL DO
PARANÁ (INSTRUÇÃO NORMATIVA Nº 01/92-CEP)

A UNIVERSIDADE FEDERAL DO PARANÁ, sediada à Rua XV de Novembro n.º 1299 Curitiba CEP 80.020-300 PR CGC 75.095.679/0001-49 Fone 3310-2656 ou 3310-2675, doravante denominada **PARTE CONCEDENTE** representada neste ato por seu **Reitor** e de outro lado, Karime Zeidan RG n.º 9.579.857 8 CPF 084304739-98 , estudante do ano/período 7º ano/13º período do Curso de Zootecnia Matricula n.º GRR20093324, residente à Rua Baltazar Carrasco dos Reis - Rebouças , n.º 1357 na Cidade de Curitiba , Estado do Paraná CEP 80230070 Fone (41) 9660-6610 / (41) 3333-9721 Data de nascimento 19/05/1991 doravante denominado (a) Estagiário (a), tendo como interveniente a Instituição de Ensino celebram o presente Termo de Compromisso em consonância com o Art. 82 da Lei nº 9394/96 - LDB, da Lei nº 11.788/08 e a Orientação Normativa nº 07/08-MPOG e com a Resolução nº 46/10 – CEPE/UFPR e mediante as seguintes cláusulas e condições:

CLÁUSULA PRIMEIRA- As atividades a serem desenvolvidas durante o Estágio constam de programação acordada entre as partes – Plano de Estágio no verso e terão por finalidade propiciar ao Estudante uma experiência acadêmico-profissional em um campo de trabalho determinado, visando:

a) o aprimoramento técnico-científico em sua formação;
b) a maior proximidade do aluno, com as condições reais de trabalho, por intermédio de práticas afins com a natureza e especificidade da área definida nos projetos políticos pedagógicos de cada curso;
c) a realização de **Estágio (X) OBRIGATÓRIO ou ( ) NÃO OBRIGATÓRIO;**

CLÁUSULA SEGUNDA - O presente estágio somente poderá ser iniciado após assinatura das partes envolvidas, não sendo reconhecido, validado e pago com data retroativa;

CLÁUSULA TERCEIRA - O estágio será desenvolvido no período de 02/03/2015 a 13/06/2015, (no prazo máximo de 02 anos), no horário das 07:30 às 12:00 e 13:30 às 15:00 hs, num total de 6 hs semanais, (não podendo ultrapassar 30 horas), compatíveis com o horário escolar podendo ser denunciado a qualquer tempo, unilateralmente, e mediante comunicação escrita, ou ser prorrogado por meio de emissão de Termo Aditivo;

Parágrafo Primeiro - Em caso do presente estágio ser prorrogado, o preenchimento e a assinatura do Termo Aditivo deverão ser providenciados antes da data de encerramento, contida na Cláusula Terceira deste Termo de Compromisso;

Parágrafo Segundo - Nos períodos de avaliação ou verificações de aprendizagem pela Instituição de Ensino, o estudante poderá solicitar à Parte Concedente, redução de carga horária, mediante apresentação de declaração, emitida pelo(a) Coordenador(a) do Curso ou Professor(a) Supervisor(a), com antecedência mínima de 05(cinco) dias úteis;

Parágrafo Terceiro - É assegurado ao estagiário, sempre que o estágio **não obrigatório** tenha duração igual ou superior a dois semestres, período de recesso de trinta dias, a ser gozado preferencialmente durante suas férias escolares, sendo permitido seu parcelamento em até três etapas, devendo ser remunerado; os dias de recesso serão concedidos de maneira proporcional, na hipótese de estágio inferior a dois semestres;

CLÁUSULA QUARTA- Na vigência deste Termo de Compromisso o Estagiário será protegido contra Acidentes Pessoais, providenciado pela Universidade Federal do Paraná e representado pela Apólice n.º 0000484 da Companhia GENTE Seguradora .

CLÁUSULA QUINTA - Durante o período de **Estágio Não Obrigatório, o estudante receberá uma Bolsa Auxilio**, no valor de ____________________, bem como auxilio transporte no valor de R$ 6,00 (seis reais/dia útil).

Parágrafo Primeiro - A comunicação referente a freqüência e rescisão deverá ser encaminhada diretamente ao DAP/PROGEPE/UFPR, sendo de inteira responsabilidade do(a) orientador(a) do presente estágio;

Parágrafo Segundo - Ocorrendo rescisão, término ou abandono do estágio, sem que haja a possibilidade de interromper o pagamento, dentro do prazo hábil fica o estagiário obrigado a devolver os valores recebidos indevidamente, referentes a bolsa e auxilio transporte, por meio de GRU – Guia de Recolhimento da União, após cálculo apresentado pelo DAP/DIP/PROGEPE, sendo esse valor devolvido em uma única vez;

Parágrafo Terceiro - Em caso de **Estágio Obrigatório,** o estagiário não fará jus a concessão de **Bolsa Auxilio, bem como Auxilio Transporte;**

CLÁUSULA SEXTA- Caberá ao Estagiário cumprir a programação estabelecida, observando as normas internas da Parte Concedente, bem como, elaborar relatório referente ao Estágio a cada 06 (seis) meses e ou quando solicitado pela Instituição de Ensino;

CLÁUSULA SÉTIMA- O Estagiário responderá pelas perdas e danos decorrentes da inobservância das normas internas ou das constantes no presente Termo de Compromisso;

CLÁUSULA OITAVA- Nos termos do Artigo 3º da Lei nº 11.788/08, o Estagiário não terá, para quaisquer efeitos, vínculo empregatício com a Parte Concedente;

CLÁUSULA NONA Constituem motivo para interrupção automática da vigência do presente Termo de Compromisso de Estágio:

a) conclusão ou abandono do curso e o fechamento de matricula;
b) pedido da Instituição de Ensino;
c) pedido do Estagiário;
d) pedido da Unidade Concedente;
e) não cumprimento do convencionado neste Termo de Compromisso;
f) pelo não comparecimento, sem motivo justificado, por mais de cinco dias, consecutivos ou não, no período de um mês, ou por trinta dias durante todo o período do estágio.

E, por estar de inteiro e comum acordo com as condições deste Termo de Compromisso, as partes assinam em 03 (três) vias de igual teor.

Curitiba, 06 / 01 / 2015.

Walter Dilay
Coordenador Geral de Estágios
Matricula SIAD 120979
UFPR/PROGRAD/CGE

PARTE CONCEDENTE – UFPR/COORDENAÇÃO GERAL DE ESTÁGIOS
(assinatura e carimbo)

Karime Zeidan
ESTAGIÁRIO
(assinatura)

ORIENTADOR(A) do Local de Estágio
(assinatura e carimbo)

Carla Forte Maiolino Molento
Méd. Vet., MSc, PhD
LABEA-UFPR
CRMV-PR 2870

COORDENADOR(A) DO CURSO
(assinatura e carimbo)
Rodrigo de Almeida Teixeira
Coordenador do Curso de Zootecnia
UFPR - Matrícula 201825

ESTÁGIO NO ÂMBITO DA UFPR

INFORMAMOS QUE O PREENCHIMENTO DO PLANO DE ESTÁGIO É OBRIGATÓRIO

Ficha nº 1 – PLANO DE ESTÁGIO
(Instrução Normativa nº 01/92 CEP)

1. **IDENTIFICAÇÃO DO ESTÁGIO:**

(X) **ESTÁGIO OBRIGATÓRIO** ( ) **ESTÁGIO NÃO OBRIGATÓRIO**

Disciplina concomitante ao estágio:

2. **DADOS REFERENTES AO LOCAL DE ESTÁGIO:**

Unidade/Departamento: Laboratório de Bem-estar Animal/Departamento de Zootecnia. Ramal: 5788

Nome do(a) Orientador(a): Carla Forte Maiolino Molento

Cargo ou função: Professora Associada

Formação Profissional: Médica Veterinária

3. **DESENVOLVIMENTO**

Atividades previstas: Trabalhar com um banco de dados do Laboratório de Bem-estar Animal (LABEA), realizar uma revisão bibliográfica sobre vocalização em bovinos na marcação a ferro quente e elaborar um artigo científico com os resultados vistos. Auxiliar em outras atividades desenvolvidas pelo laboratório.

Curitiba, 06 / 01 / 2015.

Assinatura do(a) Aluno(a): Karine Lidan

Cabe ao(a) Professor(a) supervisor(a) bem como ao(a) Orientador(a) no local de estágio, acompanhar as atividades desenvolvidas pelo Estagiário(a), na vigência do presente Termo de Compromisso, conforme .

Visto do(a) orientador(a)

Professor(a) Supervisor(a) – UFPR
(assinatura e nome por extenso)

Carla Forte Maiolino Molento
Méd. Vet., MSc, PhD
LABEA-UFPR
CRMV-PR 2870

A SER PREENCHIDA PELA COE

04. Professor supervisor – UFPR (Para emissão de certificado):

a) Modalidade de orientação: [ ] Direta [ ] Semi-Direta [ ] Indireta

b) Número de horas da orientação no período: ________

c) Número de estagiários concomitantes com esta orientação: ________

Parecer da Comissão Orientadora de Estágio (COE), em se tratando de aluno da UFPR, em ESTÁGIO NÃO OBRIGATÓRIO ____________________

Curitiba, ____/____/______ Assinatura: ____________________